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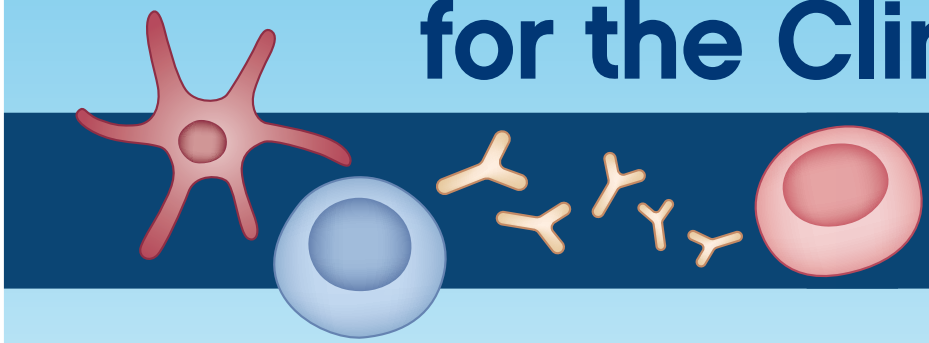

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A New *CJASN* Series: Renal Immunology for the Clinician

Fadi G. Lakkis and Paul M. Palevsky†*

Clin J Am Soc Nephrol 10: 1273, 2015. doi: 10.2215/CJN.03870415

With this issue, *CJASN* begins a new series of review articles covering immunology for the clinical nephrologist. There has been explosive growth in our understanding of immune mechanisms and the relationship between these integral defense systems within the body and the function of the kidney in health and disease. The role of the immune system as a barrier to transplantation has been long recognized and has been a primary impetus for our drive to better understand immunologic detection of nonself and mechanisms of tolerance and the development of medications to modulate the body's normal response to reject foreign organs. Immunologic dysregulation leads to the development of autoimmune kidney diseases both limited to the kidney or as part of systemic illness. These include primary glomerular diseases and interstitial nephritis as well as systemic vasculitides, collagen vascular disorders, such as SLE, and a widening array of diseases understood to be mediated by complement activation, including thrombotic microangiopathies and the spectrum of C_3 nephropathy. Increasing evidence over the past decade has also shown a central role for the immune system in the pathogenesis of AKI resulting from ischemia reperfusion injury or nephrotoxin exposure, and in sepsis, even when the kidney is not the focus of infection. An additional important connection between the kidney and immune system is the influence of CKD on immunity; paradoxically weakening defenses against infection while increasing systemic inflammation, which contributes to the excessive burden of cardiovascular disease in our patients. The role of immunologic processes in the progression of CKD is an area of growing interest.

This series, which will run over eight issues, begins this month with an overview of the immune system from an evolutionary/teleologic standpoint. In succeeding issues, the series will cover the mechanisms of the innate immune system, the normal regulation of the complement system and the role of its dysregulation in disease, the roles of dendritic cells and macrophages as both sensors and effectors in the kidney, the biology of T cells in mediating and regulating the immune response, the role of B cells, and the role of the increasing number of known soluble cytokines that allow the components of the immune system to communicate and function harmoniously. Finally, the series will end with a review of the enlarging armamentarium of pharmacologic agents at our service to control the immune response. It is the editors' hope that these reviews will serve as a primer for understanding this important and rapidly advancing field and be helpful to both seasoned practitioners and new trainees in nephrology.

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A Brief Journey through the Immune System

Karim M. Yatim and Fadi G. Lakkis

Abstract

This review serves as an introduction to an Immunology Series for the Nephrologist published in *CJASN*. It provides a brief overview of the immune system, how it works, and why it matters to kidneys. This review describes in broad terms the main divisions of the immune system (innate and adaptive), their cellular and tissue components, and the ways by which they function and are regulated. The story is told through the prism of evolution in order to relay to the reader why the immune system does what it does and why imperfections in the system can lead to renal disease. Detailed descriptions of cell types, molecules, and other immunologic curiosities are avoided as much as possible in an effort to not detract from the importance of the broader concepts that define the immune system and its relationship to the kidney.

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The Beginning of the Journey

Imagine that you are a primitive animal, perhaps a distant predecessor of all mammals. You have lived a long life, thus far relying solely on a basic defense system. If you are invaded by a microbe or parasite, you quickly expel or kill it by releasing chemicals, producing a barrage of defensive protein molecules or unleashing phagocytic cells (1). If all fails, you wall the invader off or regenerate that part of your body reduced to rot by infection. Even if infection proves fatal, your extreme fecundity, which started at a very early age, has already ensured the continuity of your species. This seemingly imaginary scenario is in fact how the more ancient of our ancestors attain near immortality (2).

Now imagine that evolution has something grander in store for you. You are destined to become the progenitor of more sophisticated beings. Your descendants will grow complex organs—robust kidneys that empower them to roam the earth and mighty brains that enable them to rule it. To do so, they will carry their embryos and nurture their young for an extended period of time. Reproduction becomes a later and infrequent event in life, and life itself becomes a much shorter journey. The capacity to regenerate tissues, limbs, and organs dwindles as tissue architecture and function grow increasingly differentiated and complex. Although less abundant, life for your descendants becomes more valuable as failure to survive until a reproductive age spells doom for the species. Faced with these burdens, you quickly realize that you have to devise a more intelligent defense system: one that protects against virtually all pathogens that your successors may encounter during their forays into known and unknown realms, one that provides long-lasting security against infection, and one that is carefully regulated so that it does not attack its own tissues or endanger beneficial cohabitants. You will call this defense system *immunity* (Figure 1). Defense, after all, is a primitive term that is equally associated with defeat and victory, whereas immunity exudes strength and confidence. So how would you (with the guiding hand of evolution, of course) go about devising such a system, what would it look like, and why will it eventually matter to our kidneys?

Innate and Adaptive Immunity

Devising a sophisticated biologic system, as evolution teaches us, does not require the destruction of preexisting, primitive tools, but instead depends on preserving and building on the best of them (3). Heeding this advice, you take your time, spending hundreds of millions of years, choosing the best and discarding the least useful of your primitive defense mechanisms. You call what is left *innate immunity*: innate because the defense mechanisms you have chosen are encoded in your germline, having been selected over evolutionary time and passed down from generation to generation with only minor refinements (4). In other words, they have stood the test of time. They include household names such as the complement system, Toll-like receptors (TLRs), and phagocytic cells. Modern-day genome sequencing has established that much of these defense systems are conserved across animal phyla, a true reflection of not only their remarkable effectiveness but also their versatility (3). A complement molecule, a TLR, or a phagocyte is not only essential for detecting and eliminating harmful nonself but is also key to maintaining normal tissue homeostasis, be it sensing and repairing damaged tissues or quietly eliminating senescent or apoptotic cells. Obviously, you have chosen prudently.

However, that is clearly not sufficient. An innate immune system provides immediate albeit incomplete protection against intruders and, at best, has only short-term memory (4,5). Instead of mounting a faster and more effective response upon encountering a known trespasser, it starts sluggishly from scratch each time. Innate immunity has additional shortcomings. Receptors utilized by innate cells, such as the TLR, are adept

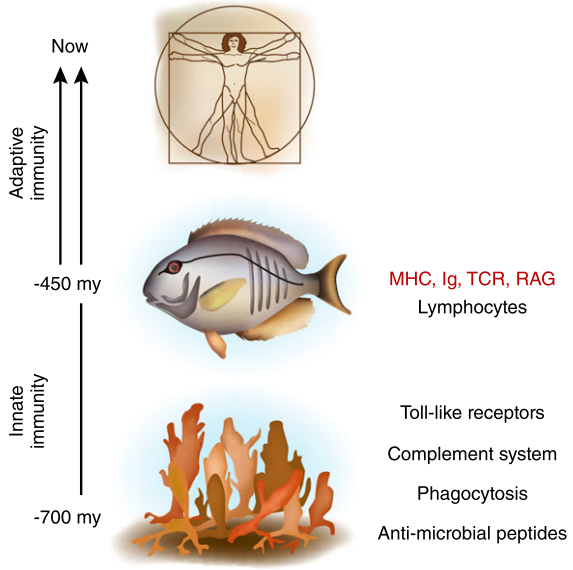

Figure 1. | **Evolution of the immune system**. Adaptive immunity as we know it in humans did not evolve until the emergence of the first jawed vertebrates (fish) around 450 million years (my) ago. Evolution of adaptive immunity was heralded by the appearance of lymphocytes, the major histocompatibility complex (MHC), immunoglobulin (Ig) molecules, T-cell receptor for antigen (TCR), and recombinase activating genes (RAG) responsible for the diversity in these recognition molecules. Our more ancient ancestors, such as the sponges (−700 my), relied on basic defense systems without the benefit of lymphocytes, antigen receptors with fine molecular specificity, or any noteworthy immunologic memory. An approximate timeline for evolution of innate immune components (antimicrobial peptides, phagocytosis, complement, and Toll-like receptors) is also shown.

at discerning self from nonself but lack the molecular specificity required for distinguishing between nonselves, causing them to trigger defenses against both friend and foe. To make matters worse, the imprecise defenses discharged by innate cells can wreak havoc on the surrounding tissue and often the entire organism itself. Imagine, by way of example, a renal abscess full of neutrophils (a typical innate cell) growing unchecked in a hapless patient.

Realizing these dangers, you set out to build a more sophisticated defense system (Figure 1). In a relatively short period (short on the evolutionary time scale, of course), you acquire the tools to create new types of immune cells, known as B and T lymphocytes (6). Lymphocytes possess surface receptors, Igs (or antibodies) on B lymphocytes, and the T-cell receptors (TCRs) for antigen on T lymphocytes that, unlike receptors on innate immune cells, recognize nonself molecules (referred to as antigens in this case) with exquisite specificity. The genes that encode these receptors are not embedded in the germline but are the product of gene recombination during lymphocyte development, a nifty molecular trick that generates a very large number of unique antigen receptors by splicing, rearranging, and linking a finite set of adjacent genes (7). Antigen receptors on either B or T lymphocytes pinpoint the slightest distinction between self and virtually any nonself or between one nonself and another and set off an immune response that only targets the antigen that happens to carry that distinction. Because only one type of antigen receptor, or perhaps a few types at most, is expressed on any given lymphocyte, this exquisite specificity ensures that only pertinent lymphocytes are activated, thus minimizing bystander damage.

However, there is more to the plan. Upon encountering antigens, lymphocytes proliferate extensively to maximize their fighting power and differentiate into specialized subsets to further hone it. B lymphocytes transform into antibody factories known as plasma cells, whereas T lymphocytes differentiate into helper and effector (*e.g.*, cytotoxic) subsets, each with its distinct set of secreted molecules (cytokines). Helper T lymphocyte subsets orchestrate the mounting immune response by dictating what defense strategy is used against a particular intruder, whereas cytotoxic T lymphocytes directly effect the death of cells harboring the intruder. Importantly, immune responses do not march on indefinitely or haphazardly but are tightly regulated by specialized B and T lymphocytes known as regulatory cells (8,9). Moreover, the exponential proliferation and differentiation of lymphocytes responding to an antigen is ultimately restrained by the death of the majority of antigen-specific lymphocytes involved in the response (Figure 2). The precious few that survive become long-lived memory cells. Memory lymphocytes ensure that a second encounter with the same invader is dealt with swiftly and effectively because of the many advantages they have over their inexperienced (naïve) predecessors (10). These include their greater number (for any given antigen), extended lifespan, more rapid response rate, superior proliferation capacity, and wider access to tissues. With the job completed, you marvel at the adaptive features of the lymphocytes you have created (clonal expansion, differentiation, regulation, and memory) and you name this new system *adaptive immunity*.

Linking Innate to Adaptive Immunity

What good, however, are two immune systems in one body if they do not communicate with each other? Because the newly devised lymphocytes of the adaptive immune system and the receptors they express are destined to recognize fine molecular specificities on antigens, you coopt the phagocytic cells of the innate immune system to capture antigens, cut them into small molecular fragments (peptides), and present them to the lymphocytes waiting in anticipation. Immunologists refer to the subset of innate immune cells proficient at processing antigens in this manner as antigen-presenting cells (APCs) and the most skilled among them as dendritic cells (DCs), because of the conspicuous dendrites they extend into every nook and cranny of our tissues (11). To activate the adaptive immune system, DCs package antigenic peptides into major histocompatibility complex (MHC) proteins (human leukocyte antigens in humans), which ensures that virtually any nonself peptide is presented to the T lymphocyte with the optimal TCR specificity and affinity (12). At the same time, DCs provide additional signals, known as costimulatory signals, which guarantee full proliferation and differentiation of the T lymphocyte (13). Parsimoniously, you choose the same molecules utilized by the innate immune system to sense nonself and trigger inflammation to be the ones that stimulate the antigen-presenting and costimulatory capabilities of DCs—that is, induce the maturation of DCs into potent APCs (14). A nonself microbial motif such as

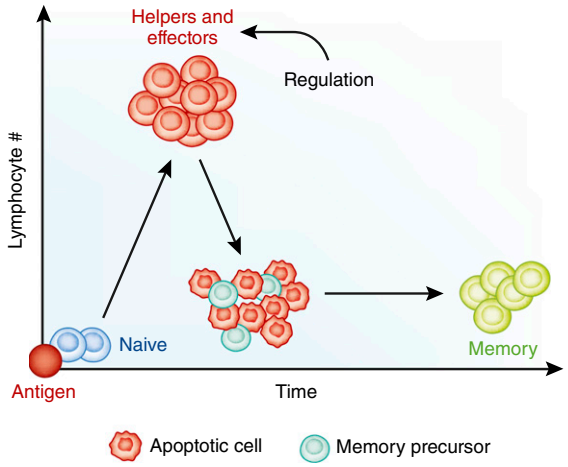

Figure 2. | A two-dimensional view of the adaptive (lymphocyte) immune response. Foreign antigen triggers the exponential proliferation of lymphocytes, which then differentiate into helper and effector cells. Regulatory mechanisms kick in at the peak of the response, the most conspicuous of which is the death of the majority of the lymphocytes by apoptosis. The few that survive become memory precursors and later memory cells. Lymphocyte death is necessary to prevent unwanted immunopathology.

lipopolysaccharide (LPS), which unleashes innate immune defenses by binding to its receptor TLR4, also primes DCs through the same receptor to present the myriad foreign antigens that the microbe carries and to activate the appropriate T lymphocytes (15). The innate immune system you have thus far molded has therefore been transformed from a primitive, first-line defense system into an ingenious doorbell that awakens the adaptive immune response (Figure 3). Adaptive immune cells, in turn, cooperate with innate immune cells—driving, fine-tuning, and sometimes regulating them—to maximize the chance that intruders are eliminated at minimal cost to the host. You congratulate yourself on successfully linking the innate and adaptive immune systems and ponder what to do next.

Lymphoid Organs: A Brief Lesson in Geography

Optimal and timely activation of the adaptive immune response, however, cannot possibly rely on chance encounters between T lymphocytes and the mature DCs that carry the antigenic peptides they recognize. Nor can random wanderings guarantee that B lymphocytes will find their target antigens or the help they need from T lymphocytes to differentiate into antibody-producing plasma cells. After all, your descendants are destined to have large bodies with extensive mucosal surfaces and complex three-dimensional organs, making the surveillance of every tissue fold by lymphocytes an impossible task. So how could one guarantee that rare immune cells find antigen and each other quickly and efficiently? The solution that evolution makes available to you turns out to be a simple one. Your descendants will harbor anatomic structures, known as secondary lymphoid organs or tissues, and will synthesize molecular messages, known as chemokines and adhesion molecules, that bring immune cells together at the right place and time (16). Prime examples of secondary lymphoid organs are the lymph nodes, spleen, and Peyer's patches in the small intestine. All are organized structures divided into T- and B-cell zones through which naïve T and B lymphocytes circulate constantly or reside for extended periods of time. DCs and other APCs, on the other hand, remain free to live in either secondary lymphoid tissues or virtually any nonlymphoid organ of the body. The kidney, in fact, has an extensive network of such cells. Upon sensing a nonself intruder and capturing its antigens (*e.g.*, an *Escherichia coli* infecting the urinary tract), DCs migrate along lymphatic channels to the nearest lymph node and, by following chemokine and adhesion molecule cues, strategically position themselves within the lymph node to activate antigen-specific T lymphocytes and subsequently, antigen-specific B lymphocytes. This organized rendezvous between innate and adaptive immune cells generates ample effector and memory lymphocytes that then exit the lymph node and migrate through the bloodstream to the site of antigen entry (*e.g.*, the infected kidney). Effector and memory cell migration to the target tissue is once again guided by chemokines and adhesion molecules and, importantly, by antigen-presenting DCs within the tissue. Some memory T lymphocytes remain in the nonlymphoid tissues as resident memory cells that guard against reinfection with the same pathogen. With the circle completed, you are confident that the noose will tighten around the intruder's neck.

In addition to secondary lymphoid tissues, evolution has set aside primary lymphoid organs dedicated to the production and education of nascent immune cells. These are the bone marrow and the thymus. The bone marrow is where both innate and adaptive immune cells are born and is the site where B lymphocytes are educated. Newborn T lymphocytes, on the other hand, receive their education in the thymus. So what is lymphocyte education all about and why is it essential? The primary goal of education is to weed out those lymphocytes that recognize self antigens (and therefore could cause harm to the organism itself) by either killing them off or inducing in them a permanent state of unresponsiveness called anergy. This education process, referred to as negative selection (17), is necessary because the specificity of antigen receptors on B and T lymphocytes arose in the first place through random, somatic gene arrangement and not through a predetermined, germline embedded route selected over evolutionary time (such as the case is with innate receptors). Therefore, unless they are carefully selected, emerging B and T lymphocyte populations would harbor an unacceptable level of self-reactivity, unleashing the "horror autotoxicus" described by Paul Ehrlich more than 100 years ago (18). Before negative selection, T lymphocytes also undergo a positive selection step in the thymus, which ensures that only those that recognize self-MHC molecules survive (19). This step is essential because TCR do not engage free-swimming peptides but ones bound to MHC molecules, implying that only those newborn T lymphocytes that express TCR with intrinsic affinity to MHC are useful. In any given individual, T lymphocytes that recognize self-MHC with a reasonable affinity are positively selected and those that engage self-MHC with either too low or too high of an affinity die—the former by neglect and the latter in the negative selection step that ensues. What emerges at the end is a mature lymphocyte repertoire that detects millions

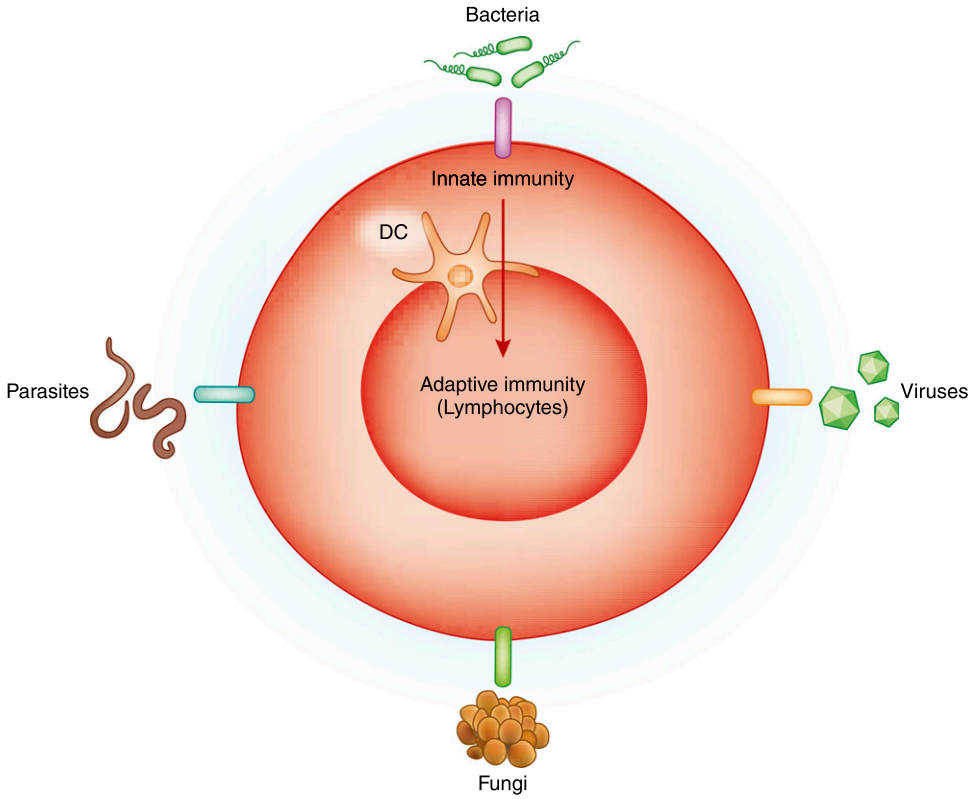

Figure 3. | **The role of the innate immune system in activating adaptive immunity**. The innate immune system can be envisioned as a doorbell that awakens the adaptive immune system (lymphocytes) upon sensing microbes (bacteria, viruses, fungi, and parasites). The dendritic cell (DC) acts as the link between the innate and adaptive systems by phagocytosing, processing, and presenting microbial antigens to lymphocytes and providing them with the necessary costimulatory signals. Endogenous molecules released by stressed or dying cells participate in acute kidney injury, transplant rejection, or autoimmunity by triggering the same innate immune receptors that sense microbes leading to stimulation of the adaptive immune response.

of nonself antigens but has a limited ability to mount an immune response against self antigens. Your heirs will be beneficiaries of this well orchestrated educational system but, as we shall see later, will also pay the price for its inherent imperfections.

Blurring the Lines

You have thus far conducted your work carefully, dividing the immune system into innate and adaptive and separating antigens along simple, clean lines into harmless self antigens on one side and harmful nonself microbes on the other. But is all self harmless, and is all nonself harmful? Are all harmful nonselves microbes? And do all immune cells fit neatly into separate innate and adaptive bins?

The immune system that you have assembled is in fact a model of versatility rather than rigid divisions. Not only do innate cells detect traditional harmful intruders (bacteria, viruses, fungi, and other pathogens), but they also respond to a subset of self-molecules (some protein and nucleic acid, others simple chemicals such as uric acid) that alarm the immune system to the presence of tissue damage (20,21). Damage-associated molecules or alarmins are released by dying or stressed cells in infected, ischemic, or injured tissues and serve two purposes: they amplify the immune response to nonself, if nonself is present as is the case with infection, and they enlist the immune system in the tissue repair process. We now know that both lymphoid (*e.g.*, regulatory T cells) and innate myeloid cells (*e.g.*, macrophages) actively participate in and are essential for tissue repair in the kidney and elsewhere (22). In other words, components of both the innate and adaptive systems are ready to react to self when it signals harm or becomes harmful itself.

Your immune system also quickly grasps the reality that not all microbial nonself is harmful. The billions of commensal bacteria and other microbes that will accompany your descendants throughout their life journeys will in fact be essential for their well-being. The immune system therefore promptly takes advantage of the regulatory mechanisms it has to ensure that DCs and lymphocytes at barrier surfaces such as the gut, skin, and lungs are carefully controlled to avoid needless attacks on helpful commensals (23). It also recognizes that nonself that is neither microbial nor pathogenic can also be harmful and must be rejected at times. Take for example a stem cell or fetus in the wrong place or potentially transmissible tumor cells (24,25). This form of nonself, known as allogeneic nonself, triggers powerful adaptive immune responses that are most apparent in the setting of transplant rejection. How lymphocytes recognize allogeneic nonself will be discussed later. How commensals interact with and shape the innate and adaptive immune systems and how the innate immune system

distinguishes between self and allogeneic nonself are not entirely clear and will surely intrigue the inquisitive minds of your descendants (26).

Finally, you come to realize that building an immune system based on inflexible distinctions between innate and adaptive immune cells is not possible (27). Evolution is not a predetermined design process; rather, it is one that advances in fits of trial and error as well as chance and necessity. Your successors will carry in them not only the final product of these efforts, a one-and-only perfect immune system, but also the marks and remnants of many immune systems. This is best exemplified, we believe, in the recent discovery of several families of innate lymphoid cells that defy traditional classification (28). On one hand, they lack antigen receptors and therefore do not display antigen specificity. On the other, they secrete classic lymphocyte cytokines and in some cases exhibit classic memory. Among innate lymphoid cells, natural killer cells pose the biggest classification challenge because they are capable of interacting with MHC molecules and generating antigen-specific immunologic memory that mirrors that of adaptive T lymphocytes (29). The precise role that innate lymphoid cells have in immunity in general and in kidney disease in particular remains to be determined.

The Immune System and the Kidney

Pondering the relationship between the kidney and the immune system brings three medical inflictions immediately to mind: autoimmune renal disease, kidney transplant rejection, and AKI (Figure 4). The first two can be thought of as mishaps or unintended consequences of the immunologic design you have put in place, whereas the third is a result of the well intentioned but sometimes overzealous response of the immune system to tissue damage. A fourth connection between the kidney and the immune system is the influence of chronic renal insufficiency on immunity. Uremia weakens crucial defenses required for protection against infection and, paradoxically, also causes generalized inflammation that is linked to excessive cardiovascular disease (30).

Autoimmune Renal Disease

The kidney can be either the direct target of autoimmunity, whereby a T lymphocyte or antibody that binds a renal antigen elicits renal pathology, or the kidney can be a victim of collateral damage caused by a systemic immune response to self or nonself antigens. In the latter setting, the culprits are usually antibody-antigen complexes (immune complexes) trapped in the glomerular filtration barrier that then instigate local inflammation (31). Autoimmunity is the consequence of the activation of those few self-reactive lymphocytes that the immune system failed to purge in the bone marrow or thymus during ontogeny. Immunologists refer to the purge as central tolerance because it takes place in central or primary lymphoid organs. Fortunately, the activation of self-reactive lymphocytes is a relatively rare event because of additional regulatory mechanisms present outside primary lymphoid organs. Immunologists refer to these as peripheral tolerance because they exert their regulatory functions in secondary lymphoid and nonlymphoid organs—that is, in the periphery. A key component of peripheral tolerance is regulatory T lymphocytes, which ensure that self-reactive lymphocytes are prevented from reacting to self or are quickly silenced if they do. Several events or circumstances, however, can lead to the breakdown of peripheral tolerance and the emergence of autoimmune disease (32). These include genetic mutations that disrupt regulatory T lymphocyte development, maintenance, or function; inflammatory events such as infection that interfere with the function of regulatory T lymphocytes; cross-reactivity between self and nonself antigens whereby T lymphocytes or antibodies specific to microbial antigens, which are readily incited during infection, also happen to bind self antigens; and finally, local tissue accidents that uncover hidden self antigens that had thus far been ignored by the immune system, neither deleted in the process of central tolerance nor regulated in the periphery. Finally, because of its key function in blood filtration, the kidney is often the resting place for antigen-antibody complexes that form elsewhere or sometimes locally after antigen is trapped in the glomerulus (31). Immune complexes can either be the result of a systemic autoimmune process (*e.g.*, SLE) or the product of an immune response to microbes (as may be the case in glomerulonephritides that arise after infection). In both cases, complement activation by antibody molecules appears to play a major role in triggering renal pathology, but the full armamentarium of the immune system, including innate and adaptive cells as well as the cytokines they produce, participates. Through no fault of its own, the kidney obviously can be the target of the wrath of immunity.

Transplant Rejection

Another price that your descendants will pay for the highly sophisticated but imperfect immune system you have bestowed upon them is the rejection of life-saving organ transplants. In the absence of any immunosuppressive drugs, a kidney transplanted from one human to a genetically disparate human (*i.e.*, someone who is not an identical twin with the donor) will be rejected violently. The rejection process is dependent on T lymphocytes, although all other immune defenses participate in one way or another in the rejection process, and the T lymphocyte response to the transplanted organ (the allograft) is characterized by sheer immensity that far exceeds any antimicrobial response (33). So why are T lymphocytes strongly alloreactive if natural selection has indeed been busy perfecting the repulsion of harmful pathogens, not harmless organ transplants? The answer lies first in the fact that any given individual harbors a large number of T lymphocyte clones that recognize and react to MHC antigens, which are the principal histocompatibility antigens responsible for transplant rejection; second, many of these T lymphocyte clones have already acquired memory properties (34). The large number of T lymphocytes that react to MHC antigens is a byproduct of an immune system that selects its T lymphocytes based on their ability to recognize peptides bound to MHC molecules (35). The memory nature of many of the alloreactive T lymphocytes is because TCRs specific for a microbial peptide (presented in the context of self-MHC) are also capable of recognizing allogeneic, nonself MHC—that is, they are cross-reactive (36). For example, memory T lymphocytes generated after exposure to a ubiquitous virus such as the Epstein–Barr virus cross-react with allogeneic MHC molecules and cause vigorous transplant rejection. Therefore, in

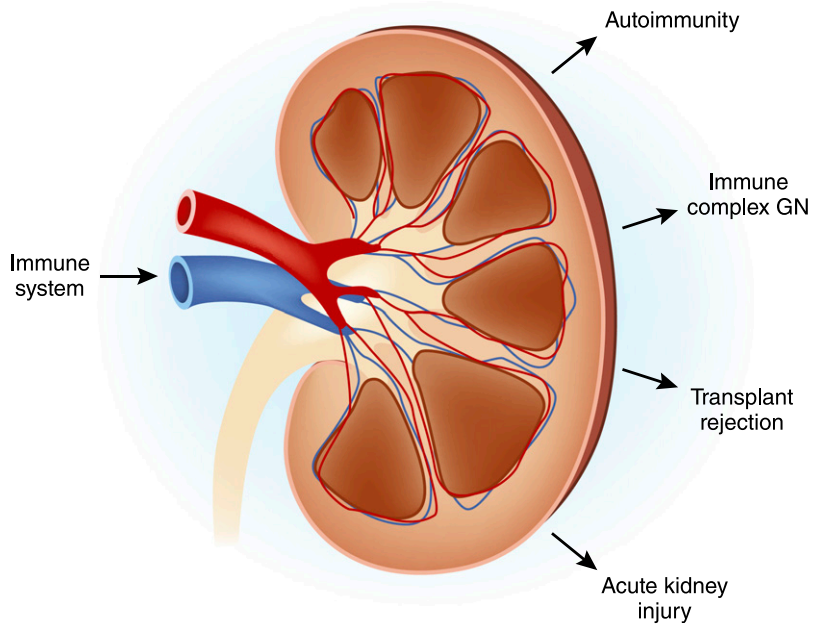

Figure 4. | **The relationship between the immune system and kidney disease**. The principal renal afflictions in which the immune system plays a major or important role are shown. Conversely, renal insufficiency affects the immune system by weakening immune defenses and by causing systemic inflammation that contributes to cardiovascular disease.

its obsession to create an immune system that is able to respond to practically any pathogen, evolution put in place a highly diverse (polymorphic) MHC system that can bind virtually any microbial peptide and present it to T lymphocytes, whose TCRs to begin with are biased to bind to and sample all sorts of MHC molecules. Neither you nor evolution, it appears, predicted that some of your descendants will become talented transplant surgeons and nephrologists and that the polymorphic MHC proteins that are essential for antimicrobial immunity will also act as a powerful histocompatibility barrier to organ transplantation.

AKI

A less anticipated and, until recently, overlooked function of the immune system is its role in tissue injury unrelated to infection—so-called sterile tissue injury. AKI, which is the end result of a variety of noninfectious insults such as ischemia, drugs, and toxins, is often accompanied by subtle infiltration of the kidney with leukocytes from the blood and not-so-subtle activation of intrarenal immune cells. The infiltrate is not restricted to innate, myeloid cells (neutrophils and monocytes, for example) but also includes lymphoid cells, both adaptive and innate (37). Similarly, activation of renal cells involves resident macrophages and DCs as well as renal epithelial cells. The latter are increasingly recognized as accomplices of the immune system because they express innate receptors such as the TLR, respond to TLR ligands, and produce a host of inflammatory and immune cytokines (38). The net sum of immune activation after AKI, however, is still puzzling. On one hand, it can lead to more harm by causing excessive inflammation; on the other, it can be beneficial by repairing damaged tissues and cleaning up the mess (31,39). If nephrologists could uncover the secret to striking the right balance, immune therapy of AKI will one day become a reality (40).

Epilogue

It is not often that one biologic system touches so many aspects of human biology in both sickness and health. Although it is seemingly esoteric and beyond comprehension at first blush, the immune system, once viewed through the prism of evolution, is the epitome of versatility and simplicity of purpose. By peeling its layers one at a time, immunologists have succeeded not only in elucidating the inner workings of immunity but have also enabled the translation of their discoveries into real life benefits, such as vaccines that eradicate scourges, immunosuppressive drugs that conquer allograft rejection, cytokine-based therapies that subdue autoimmune disease, and antibodies that unbridle T lymphocytes to attack cancer cells. However, there is still much left for us nephrologists to do and discover. Which immunologic pathways should we target to interrupt or reverse GN? Of the many T lymphocyte, B lymphocyte, cytokine, and complement-based treatments that are now available in the clinic, which ones should we test in our patients? How can we improve long-term renal allograft outcomes without further compromising the immune system and therefore the health of the transplant recipient? What have we missed at a fundamental scientific level that still prevents us from achieving immunologic tolerance to autoantigens or organ transplants in a safe and effective manner, sparing patients the unwanted consequences of global immunosuppression? What immunologic trick can we pull to combat AKI? The list goes on and on as far as the imagination can see. The real journey has only begun.

Glossary

Adaptive Immunity

Adaptive immunity comprises defense mechanisms mediated by immune cells known as lymphocytes (T, B, and natural killer cells) and the specialized molecules required for their function. The term *adaptive* is applied because lymphocytes rapidly adapt to the situation at hand (*e.g.*, a specific type of microbial infection) generating specialized cells, cytokines, and antibodies as well as long-lasting immunologic memory.

Antigen

Antigen is a nonself molecule, usually a protein, that incites an adaptive immune response.

Cytokines

Cytokines are protein molecules produced by cells of the immune system that mediate diverse defensive functions. These include inflammation, lymphocyte activation and differentiation, and killing of cells harboring foreign antigens. Cytokines play an important role in the pathogenesis of autoimmunity and immune-mediated renal disease.

Dendritic Cells (DCs)

Dendritic cells (DCs) are a specialized myeloid cell that is induced by infection to take up antigens, process them into small peptides, package them inside major histocompatibility complex (MHC) molecules, and present them to T lymphocytes after migrating to secondary lymphoid organs. DCs are prototypical antigen-presenting cells (APCs). They link innate to adaptive immunity.

Innate Immunity

Innate immunity comprises defense mechanisms mediated by the evolutionary more primitive components of our immune system. These include myeloid cells such as macrophages, DCs, and neutrophils and protein molecules such as the complement and coagulation systems. The term *innate* is used because these defenses are hardwired in the genome, responding in a rather unvarying manner to injury or infection. The innate immune system activates the adaptive immune system, principally *via* antigen-presenting DCs.

Lymphocytes

Lymphocytes are hematopoietic cells that mediate adaptive immunity. B lymphocytes produce antibodies (humoral immunity), whereas T lymphocytes differentiate into the specialized subpopulations best suited to tackle the offending agent (cellular immunity).

Major Histocompatibility Complex (MHC)

The MHC is a gene complex that codes for a diverse group of related protein molecules expressed on all nucleated cells (in the case of class I MHC molecules) and on immune cells (in the case of class II MHC molecules). In humans, they are known as the human leukocyte antigens. MHC molecules bind antigenic peptides, making possible their detection by T lymphocytes. Therefore, they are necessary for initiating adaptive immune responses. Because of their great diversity in humans, MHC molecules also act as histocompatibility antigens that trigger the rejection of transplanted organs.

Primary Lymphoid Organs

Primary lymphoid organs are organs or tissues where lymphocytes are born and/or trained to recognize and react to nonself antigens but not self-molecules. They include the bone marrow and the thymus.

Secondary Lymphoid Organs

Secondary lymphoid organs are organs or tissues where mature (trained) lymphocytes reside or circulate through. They are the site where lymphocytes encounter antigens and are activated by them to produce antibodies or effector (fighter) cells. Secondary lymphoid organs include the spleen, lymph nodes, and mucosal lymphoid tissues such as the Peyer's patches in the small intestine.

Toll-Like Receptors (TLRs)

Toll-like receptors (TLRs) are receptors expressed principally on innate immune cells but also present on adaptive immune cells and nonimmune cells. They detect conserved molecular patterns on microbes. The TLR4 receptor, which binds lipopolysaccharide (LPS) of Gram-negative bacteria, is a prototypical example. TLRs also sense tissue damage by binding endogenous molecules released by dying or stressed cells. TLR engagement triggers inflammation as well as DC maturation, leading to enhancement of the adaptive immune response.

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Disclosures

None.

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How the Innate Immune System Senses Trouble and Causes Trouble

Takashi Hato and Pierre C. Dagher

Abstract

The innate immune system is the first line of defense in response to nonself and danger signals from microbial invasion or tissue injury. It is increasingly recognized that each organ uses unique sets of cells and molecules that orchestrate regional innate immunity. The cells that execute the task of innate immunity are many and consist of not only "professional" immune cells but also nonimmune cells, such as renal epithelial cells. Despite a high level of sophistication, deregulated innate immunity is common and contributes to a wide range of renal diseases, such as sepsis-induced kidney injury, GN, and allograft dysfunction. This review discusses how the innate immune system recognizes and responds to nonself and danger signals. In particular, the roles of renal epithelial cells that make them an integral part of the innate immune apparatus of the kidney are highlighted.

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Introduction

The innate immune system is the first line of defense against infection (nonself) or tissue injury (damaged self). The cells and molecules of innate immunity are rapidly activated by encounter with microbes or other "danger signals." The rapidity of the response is essential because of the fast doubling time of typical bacteria. The innate immune system was once perceived as a crude stopgap until the adaptive immune system activates. It is now understood that innate immunity is a highly sophisticated sentinel system vital to maintaining a healthy tissue microenvironment. In fact, the innate immune system first appeared 750 million years ago and has been remarkably conserved throughout the evolutionary tree of life. To put it into perspective, the rodent and human lineage separated from a common ancestor only 80 million years ago (1–3).

The components of the innate immune system are many. They include soluble recognition molecules, such as natural antibodies, pentraxins (*e.g.*, C-reactive protein), and the complement system. Cellular components of the innate immune system consist of phagocytic cells (*e.g.*, macrophages), antigen presenting cells (*e.g.*, dendritic cells), and killing cells (*e.g.*, natural killer cells). In addition, subsets of T and B cells have limited antigen receptor diversity and also participate in innate immunity (*e.g.*, invariant natural killer T cells, $\gamma\delta$ T cells, B-1 B cells). Finally, epithelial cells are an integral component of innate immunity and function as physical barriers, producers of cytokines and chemokines and have the ability to actually recognize and process danger signals. Although epithelial cells are generally viewed as unofficial members of the professional immune system, they constitute the vast majority of cells in a given organ, and, therefore, their relative contribution to immunity can be substantial.

In this review, we first discuss how the innate immune system recognizes and responds to danger signals in general. We then shift the focus to the kidney. In particular, we highlight the roles of renal epithelial cells as important trouble sensors and possibly trouble makers. This epithelial cell–centric view, which is an important concept in the danger model, was first proposed by Polly Matzinger (4–6).

> The danger model says that it is a tissue that controls whether you turn on an immune response, by sending alarm signals. It is also a tissue that induces tolerance by allowing its antigens to be presented without alarm signals. Perhaps, therefore, it could also be the tissue that determines the class of immunity.

How Cells Recognize and Respond to Danger Signals

Bruce Beutler's seminal discovery of the endotoxin receptor, Toll-like receptor (TLR) 4 (TLR4), in 1998 revolutionized our understanding of innate immunity (7). We now know that most mammalian species have 10–13 types of TLRs and that each receptor recognizes specific ligands and induces a wide array of inflammatory cascades (8) (Figure 1). TLRs are expressed most heavily in myeloid-lineage cells but are also found in other cell types, including renal epithelial cells (9–13). We discuss the roles of TLRs in renal epithelial cells later in this review.

Structurally, all TLRs are membrane-bound glycoproteins and have characteristic ligand-binding motifs (leucine-rich repeats and cysteine-rich repeats) and cytoplasmic signaling domains (Toll/IL-1 receptor [TIR] homology domains) (8). TIR domains are also found in cytokines, such as IL-1 and IL-18, and therefore

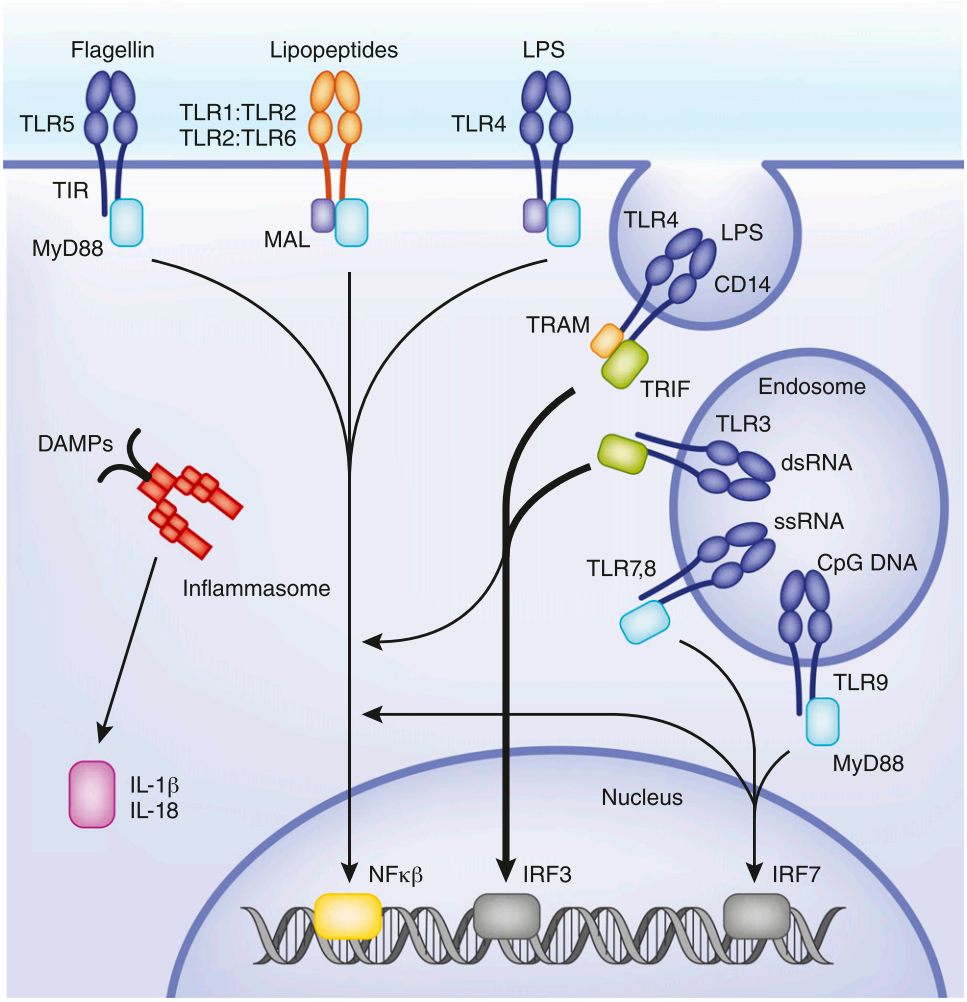

Figure 1. | **Location and signaling pathways of pattern recognition receptors**. Toll-like receptors (TLRs) are membrane-bound glycoproteins and consist of a functional homomer (*e.g.,* TLR4) or heteromer (*e.g.,* Toll/IL-1 receptor [TLR] 1:TLR2). TLRs have characteristic ligand-binding motifs (leucine-rich repeats and cysteine-rich repeats) and cytoplasmic signaling domains (TIR homology domains). Note the differential localization of TLRs. Upon activation of TLRs, the TIR domain engages the adaptor molecule MyD88, with the exception of TLR3, which exclusively signals through TRIF. The TIR domain of TLR4 can engage both MyD88 and TRIF pathways. The coreceptor CD14 facilitates internalization of TLR4 and subsequently activates TRIF signaling pathway. The best-characterized cytosolic receptor is the NLRP3 inflammasome complex. The mature inflammasome activates caspase-1, which in turn generates IL-1β and IL-18. These cytokines induce various proinflammatory pathways, including programmed inflammatory cell death (pyroptosis). CpG DNA, unmethylated cytosine-phosphate-guanine DNA; DAMPs, damage-associated molecular patterns; dsRNA, double-stranded RNA; IRF, IFN regulatory factor; MAL, MyD88-adapter–like; MyD88, myeloid differentiation primary response gene 88; NLRP3, NOD-like receptor family, pyrin-domain-containing 3; ssRNA, single-stranded RNA; TRAM, Toll-like receptor 4 adapter protein; TRIF, TIR domain–containing adapter-inducing INF-β.

share similar signaling pathways leading to inflammation. Upon activation, TIR domains engage the adaptor molecules myeloid differentiation primary response gene 88 (MyD88) or TIR domain–containing adapter-inducing INF-β (TRIF). TLR3 signals exclusively through TRIF while other TLRs signal primarily through MyD88. TLR4 is unique in that it can activate both MyD88 and TRIF pathways (Figure 1). In addition to the membrane-bound TLRs, many cytosolic receptors have also been discovered over the past decade (14). The two major classes of the cytoplasmic receptors are Nucleotide-binding oligomerization domain-like receptors (NOD-like receptors, NLR) and retinoic acid-inducible gene-I-like receptors (RIG-like receptors, RLR). In particular, the cytoplasmic signaling complexes, commonly called inflammasomes, are under intense investigation (15–19).

These membrane-bound and cytosolic receptors are collectively called pattern recognition receptors (PRRs) because they recognize specific structural patterns. The specificity is remarkable, reminiscent of adaptive immunity. However, the specificity of innate immunity differs from that of adaptive immunity in several aspects (Table 1) (2,20). The innate immune system recognizes structures shared by classes of microbes, whereas adaptive immunity recognizes individual details of microbes (antigens). The microbial structures recognized by innate immunity, called pathogen-associated molecular patterns (PAMPs), are characteristic of microbes

Table 1. Characteristics of innate and adaptive immunity

Innate Immunity	Adaptive Immunity
Initial response (hours)	Later response (days)
Recognizes microbial nonself, molecular patterns unique and often essential to microbes (PAMPs)[a]	Antigen-specific response; recognizes individual molecular details (6–30 amino acid residues) derived from microbes or self
Receptors are encoded in germline	Receptors are generated by somatic recombination
Nonclonal	Clonal expansion
No memory	Memory
Limited diversity	Large diversity
Cells: phagocytic cells (*e.g.*, macrophages, neutrophils), natural killer cells, antigen presenting cells (*e.g.*, dendritic cells), and epithelia (physical barrier)	Cells: T, B lymphocytes
Components: TLR, NLR, RLR, scavenger receptor, N-formyl methionyl receptor, C-type lectin-like receptor (*e.g.*, mannose receptor), soluble recognition molecules (*e.g.*, pentraxins, complement, natural antibodies).	Components: TCR, BCR, antibodies

PAMPs, pathogen-associated molecular patterns; TLR, Toll-like receptor; NLR, NOD-like receptor; RLR, RIG-like receptor; TCR, T-cell receptor; BCR, B-cell receptor.
[a]Innate immunity also recognizes damaged-self and allogeneic non-self. See text.

but not common to the host. For example, TLR9 recognizes hypomethylated cytosine-guanine DNA sequences, which are present in microbial genomes but are uncommon or masked in mammals. In contrast, antigens recognized by adaptive immunity may not be unique to microbes. Another difference is that structures recognized by the innate immune system are often essential for survival of the microbes (*e.g.*, LPS, the essential component of the Gram-negative bacterial cell wall). Conversely, antigens recognized by adaptive immunity are not necessarily essential for survival. In fact, certain pathogenic microbes can mutate antigens to evade host adaptive immune defense without compromising their own survival. Finally, because PRRs are encoded in the germline (as opposed to somatic recombination in adaptive immunity), the number of molecular patterns that the innate immune system can recognize is limited. Nevertheless, it is estimated that innate immunity can recognize up to 10^3 molecular patterns (the adaptive immune system is estimated to recognize 10^7 or more antigens) (20,21).

One notable feature of pattern recognition receptors is their strategic location in various cellular compartments, allowing them to sense distinctive PAMPs and trigger specific downstream signaling cascades (22,23). For instance, host nucleotides are not normally present in endosomes, whereas microbial nucleotides can be found in endosomes following phagocytosis. Therefore, endosomal distribution of TLR3, 7, 8, and 9 (receptors of nucleotides) will allow the host to respond to microbial nucleotides but not to host nucleotides (Figure 1).

The fact that pattern recognition receptors recognize structures shared by broad classes of microbes poses a dilemma. How does the host discern pathogenic microbes from nonpathogenic microbes? This is not trivial; the number of bacteria we host amounts to 10^{14}, 10 times more than all the human cells in one individual. Most of these bacteria are harmless or even beneficial (commensals). However, they are also equipped with the same microbial structures found in pathogenic strains, such as LPS. How the innate immune system distinguishes the good from the bad remains an intense area of research as it relates to broad clinical problems, such as allergy and chronic inflammatory diseases. Medzhitov, who cloned the human TLR4, figuratively describes it: "Detecting a person in a building does not necessarily mean they are an intruder, since not all people are intruders. But if someone comes into the building through a window at night, then that might indicate the person is a burglar" (24).

So, perhaps not surprisingly, PRRs expressed on sentinels such as macrophages can also recognize "damaged self" and trigger inflammation. Typically, sentinels see "damaged self" by sensing endogenous soluble molecules that are confined within the cell under normal state but are released after injury. The prototypes of the endogenous molecules include extracellular ATP, high-mobility group box protein 1, and heat shock protein, collectively called damage-associated molecular patterns (DAMPs) (25). DAMPs can induce strong inflammation and the net clinical outcomes are often indistinguishable from those of PAMP-induced inflammation. Indeed, sterile-tissue injury, such as blunt trauma, results in a "genomic storm" that highly resembles endotoxin-induced transcriptome changes (26). DAMPs are also highly relevant in the settings of renal ischemia-reperfusion and allograft injury (27). Of note, some DAMPs do not directly bind to their PRRs. Instead, these DAMPs are believed to induce small structural changes in other molecules that activate the receptor and its downstream pathway (28).

Upon activation, PRRs can induce three major types of responses: (*1*) phagocytosis, (*2*) inflammation, and (*3*) maturation of antigen-presenting cells (*e.g.*, macrophages and dendritic cells), which leads to activation of the adaptive immune system (Figure 2) (29). The cellular and molecular details of these responses are extensively covered in general immunology reviews (8,30–32). Notably, the maturation of

antigen-presenting cells provides an important link between the innate immunity and adaptive immunity. It is important here to point out that PAMPs are not necessarily the final antigen being presented by antigen-presenting cells. PAMPs do activate their cognate PRRs and initiate phagocytosis, but the final modified and presented antigen is likely another constituent of the phagocytized microbe. The biology of antigen capture and presentation has attracted and will continue to captivate scientists because it encompasses the most fundamental question of immunology: self/nonself discrimination (29).

Phagocytosis is a platform for activation of many PRRs and often a prerequisite for activation of inflammatory signaling cascades. For example, CD36, a scavenger receptor expressed on phagocytic cells, recognizes microbial diacylglycerides and prompts phagocytosis. This in turn leads to proinflammatory responses. Ideally, the inflammatory responses should confine infection and improve the host outcome. Unfortunately, excessive inflammation often results in collateral tissue damage. Indeed, it has been reported that the inhibition of CD36 reduces inflammation and even improves the survival rates in an animal model of sepsis despite the impaired scavenging function (33).

Clinically, the inflammatory cytokine storm results in vasodilation, refractory hypotension, and ultimately death. At the cellular tissue level, various degrees of oxidative stress, cell cycle arrest, and damaged organelles (*e.g.*, mitochondria) can be observed in various organs, including the kidney (34–38). To mitigate the cytokine storm, many clinical trials have sought to block PRRs in patients with severe infection. The most illustrative example is the inhibition of TLR4. Eritoran, an inhibitor of TLR4, was thought to be effective in reducing sepsis-induced mortality by blocking inflammation. Contrary to expectations, multiple clinical trials have failed to demonstrate positive outcomes with TLR4 inhibition (39–41).

To some, the failure of TLR4 inhibition was not unexpected. It has long been known that TLR4 mutant mice are resistant to endotoxin yet are highly susceptible to gram-negative bacterial infection because they cannot sense or react to actual bacterial invasion (7). This raises an important clinical question: the balance between elimination of microbes and minimizing inflammation. Could we find a compromise whereby killing of microbes, although not perfect, may involve minimal collateral tissue damage? Emerging data suggest that it is possible for the host to do so (42,43). The interested reader is referred to Jamieson and colleagues' recent article, which also points to the importance of tissue repair capability (44).

How the Innate Immune System Senses Trouble and Causes Trouble in the Kidney

Renal epithelial cells are surrounded by a dense network of macrophages and dendritic cells, collectively called mononuclear phagocytes. These mononuclear phagocytes are thought to play an important role in maintaining the integrity of tissue microenvironments. In fact, mononuclear phagocytes are abundantly present even in early embryonic kidneys (45). Mononuclear phagocytes have markedly diverse functions: from traditional phagocytic function and inflammation to versatile, trophic roles. We do not go into the details of renal mononuclear phagocytes because this is covered by Kurts *et al.* in this *CJASN* Immunology Series. Instead, here we focus on the often underappreciated roles of renal epithelial cells in sensing danger signals.

Many PRRs, including TLRs, are expressed in renal epithelial cells (46–54). The precise distribution of tubular TLRs remains somewhat uncertain. This is in part due to the inherent complexity of the kidney architecture. One needs to combine technically intricate microdissection, *in situ* hybridization, and immunostaining to adequately characterize TLR

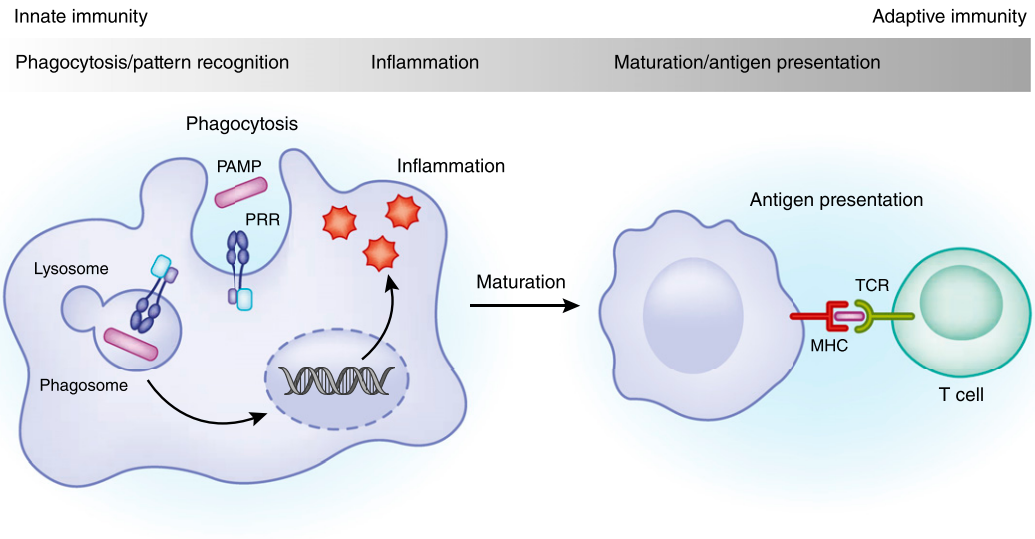

Figure 2. | **Innate immune responses encountered by microbes**. Microbes are detected by pattern recognition receptors (PRRs) expressed in innate immune cells, such as macrophages.The detection of microbes by the PRRs rapidly activates signaling cascades and generates inflammatory responses. Microbial encounter also leads to maturation of macrophages and dendritic cells into antigen presenting cells. PAMP, pathogen-associated molecular pattern; TCR, T-cell receptor.

expression and distribution among various renal cell populations. In this regard, immunostaining remains challenging because of lack of firm antibodies in this class. Moreover, TLRs are such potent receptors that the expression levels are typically low at the levels of mRNA and protein. In monocytes, it is estimated that TLR4 is present at 1300 molecules per cell, whereas CD14, the coreceptor of TLR4, is expressed at 115,000 molecules (55). In nonmyeloid cells, TLR4 expression is likely much lower. Nevertheless, because the total number of epithelial cells far exceeds that of immune cells, tubular TLRs are an important part of renal innate immunity. In support of this, Wu *et al.* performed a classic experiment (56). They examined the effect of renal ischemia-reperfusion injury in bone-marrow chimeric mice between TLR4 knockout and wild-type animals. Chimeric mice lacking intrinsic renal TLR4 had significantly less tubular damage and azotemia than mice lacking hematopoietic TLR4, indicating that TLR4 in the kidney is instrumental in mediating tubular damage. Using a model of endotoxemia, we also demonstrated that endotoxin-induced tubular injury has an absolute requirement for tubular TLR4 (57). Conversely, TLR4-expressing hematopoietic cells were not essential or sufficient to cause tubular toxicity. Zhang *et al.* and Pulskens *et al.* also showed the importance of intrinsic renal TLR4 after cisplatin nephrotoxicity and ischemic injury, respectively (58,59). Similarly, Leemans *et al.* examined bone-marrow chimeric mice between TLR2 knockout and wild-type mice and found that intrinsic renal TLR2 has a central role in the unfolding of the injury process (60). In summary, collective evidence strongly indicates that epithelial TLRs contribute to tissue injury and inflammation in response to danger signals.

In human kidney transplantation, Kruger *et al.* reported differences in TLR4 expression in kidney tubules from deceased versus live donors (61). The same authors also identified loss-of-function single-nucleotide polymorphisms, Asp299Gly and Thr399Ile, in TLR4 genotype in a large cohort of donors (62,63). These kidneys with a TLR4 loss-of-function allele had a higher rate of immediate graft function. Although hematopoietic TLR4 likely contributed to inflammation to some extent, this study highlights the significance of renal tubular TLR4 in graft function. Detailed reviews on the role of TLRs in renal allograft can be found elsewhere (64,65).

From a methodologic standpoint, a limitation of these transplant and bone-marrow chimera approaches is that results could be confounded by other nonimmune, nontubular cell types, such as endothelium. Therefore, studying animals with cell type–specific gene manipulation may further illuminate the roles of TLRs in each cell type. In this regard, Deng *et al.* conducted an interesting study in the liver in which they deleted TLR4 from hepatocytes or myeloid cells. They found that hepatocyte TLR4 plays an important role in clearing endotoxin and limiting sepsis-induced inflammation and organ injury (66).

Could renal epithelial TLR4 also be playing a role in endotoxin clearance? Bacterial endotoxin can be filtered through nephrons and taken up by the proximal tubules. Specifically, we found that endotoxin undergoes TLR4-mediated endocytosis in S1 tubular segments (Figure 3) (55). Like professional phagocytes, S1 tubules exhibited autoprotection that was in part mediated by upregulation of antioxidant and cytoprotective pathways (67). As such, S1 segment acts as the "sensor" and "sink" of endotoxin in the filtrate and can initiate signaling to adjacent segments, such as S2 and S3. However, with large endotoxin exposures, this signaling manifested as widespread oxidative stress in these downstream segments. These findings indicate that S1 segments may

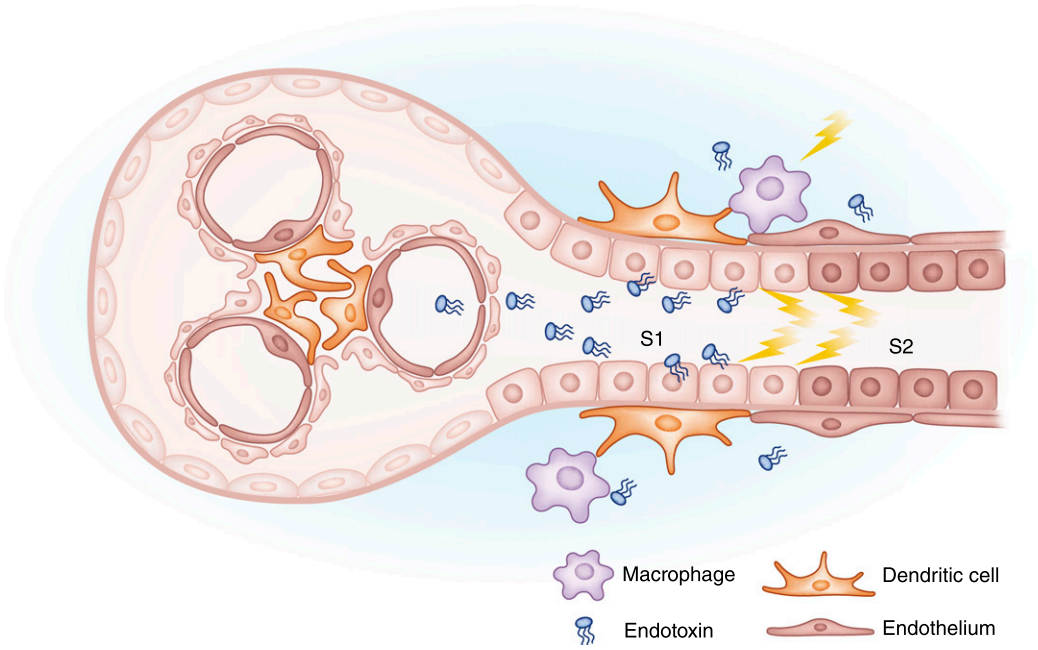

Figure 3. | **A model of endotoxin-induced tubular injury.** Endotoxin, released from bacteria in various molecular sizes, can be filtered through nephrons and internalized by S1 proximal tubules through a Toll-like receptor 4–dependent mechanism. The interaction between endotoxin and S1 can result in oxidative stress and injury in downstream tubular segments. Yellow lightning bolts represent signaling molecules released by macrophages or S1 cells after interacting with endotoxin.

play a sentinel role similar to macrophages and could be considered as an epithelial macrophage, or "epiphage."

Besides generating inflammation, phagocytosis is another hallmark of mononuclear phagocytes. Ichimura *et al.* demonstrated that kidney injury molecule-1, a proximal tubule injury marker, is a phosphatidylserine receptor and as such can function as a scavenger receptor (68). Therefore, during tubular injury, proximal tubular cells are transformed into "semiprofessional phagocytes" (68). This further illustrates the principle of shared functions between epithelial cells and professional innate immunity. Furthermore, MHC II and costimulatory proteins can be expressed on proximal tubules after various stimuli, and some data even suggest that proximal tubules could present antigens to T cells (69–76). Distal tubules also express PRRs and participate in local immune responses (77–80). One important difference remains between epithelial cells and professional innate immunity: mobility. Renal epithelial cells do not typically translocate. Therefore, epithelial cells alone will not be able to accomplish higher levels of immune activities (such as remote information transfer) unless they are supported by immune cells. Ultimately, epithelial cells and immune cells are both essential in shaping renal immunity. With advances in multiplexed, single-cell technologies and ever-increasing genetic tools (81–83), we anticipate that many exciting discoveries will be made at the cellular and molecular levels and will elucidate the mechanisms of epithelial cell–immune cell communication.

We have discussed recent advances in our understanding of renal innate immunity with special emphasis on renal epithelial cells. However, this epithelial cell–centric view should not preclude the contribution of other nonimmune cells to overall renal innate immunity. For example, there is a wealth of literature suggesting that certain types of glomerular injury are mediated by PRRs expressed on podocytes (84). It is proposed that proinflammatory cytokines generated from glomeruli could spread inflammation along the tubules through peritubular capillaries (85). Heightened PRR activation in the endothelium is another important source of inflammation (86,87), while properly activated endothelium is critical for mobilizing immune cells and clearing microbes (88). We also point out that because of the sentinel nature of innate immunity, studies have primarily focused on acute pathologic changes rather than long-term consequences of PRR activation, such as its role in fibrosis (89–91). From a clinical perspective, several kidney diseases have been linked to deregulated innate immunity and inflammation (Table 2) (92–94). For example, Mulay *et al.* demonstrated that tubular injury from calcium oxalate crystals is triggered by NLRP3 inflammasome in renal mononuclear phagocytes (95). In both human IgA nephropathy and an animal model of IgA nephropathy, recent genome-wide association studies identified susceptibility polymorphisms involved in innate immunity and inflammation (96,97). In fact, a more recent investigation of gene expression variants by expression quantitative trait loci analysis revealed a high degree of overlap between SNPs important in regulation of innate immunity and those associated with renal disease phenotypes (98).

Table 2. Kidney diseases and innate immunity

Disease or Condition	Molecules Involved	Comments	Reference
IgA nephropathy	Defensin, TNFSF13	Human, GWAS	96
	TLR9, MyD88	Murine (ddY[a]), GWAS	97
Diabetic nephropathy	TLR4	Human	93
Kidney transplant	TLR4, CD14, TLR3	Human, polymorphisms	61,102–105
	MyD88	Murine	106
Renal disease[b]	LPS-stimulated molecules	Human	98
GN	TLR4, TLR2	Murine (TSLP/Fc_{γ}RIIb[a], nephrotoxic serum)	84,107,108
Hepatitis C–associated GN	TLR3	Human	109
Lupus nephritis	MyD88, TLR7, TLR9	Murine (MRL/*lpr*[a])	110–112
Nephrocalcinosis	NLRP3	Murine (calcium oxalate crystals)	95
Cisplatin nephrotoxicity	TLR4	Murine	59
Urinary obstruction	TLR4	Murine	90
Polycystic kidney disease	CD14	Murine (cpk[a])	113
Urinary tract infection	TLR4, TRIF, SIGIRR	Human	114
	TLR4, TLR5, TLR11	Murine (E-coli)	77,78,115–118
Proteinuria	CD80, TLR4	Murine (LPS)	119
Sepsis-induced AKI	TLR4, TLR2, TLR9, MyD88	Murine (LPS, CLP)	57,120–123
Ischemia-reperfusion injury	TLR4, TLR2, CD14, NLRP3, Nod1, Nod2	Murine	53,56,58,60,86,94,124–127

TNFS13, TNF ligand superfamily member 13; GWAS, genome-wide association study; MyD88, myeloid differentiation primary response gene 88; eQTL, expression quantitative trait loci; NLRP3, NOD-like receptor family, pyrin-domain-containing 3; SIGIRR, single immunoglobulin IL-1-related receptor; CLP, cecal ligation and puncture.

[a]Animal models for the indicated diseases.

[b] Enrichment of eQTL by GWAS ontology category "renal disease".

We address now the more complex issue about the transition from innate to adaptive immunity. Indeed, a full innate immune response is expected to culminate in the maturation of antigen-presenting cells and the triggering of adaptive immunity. An important question therefore relates to the equivalence of DAMPs and PAMPs in that regard. That is, are DAMPs capable of eliciting a full innate immune response beyond causing local inflammation through their interactions with PRRs? A recent study by Oberbarnscheidt *et al.* suggests that this might not be the case. Indeed, these authors showed that DAMPs released from ischemic injury to syngeneic grafts were not sufficient to cause full antigen-presenting cell maturation and adaptive immunity. Conversely, an allogeneic graft, similarly subjected to ischemic injury, did trigger a full innate immune response and activated adaptive immunity. This suggested that, beyond DAMPs, innate immune cells could also be sensing allogeneic nonself (allorecognition), a property previously thought to exist only in adaptive immune cells. The authors proposed that it was the recognition of allogeneic nonself rather than DAMPs that linked innate immunity to adaptive immunity and thus offered a unification of alloimmunity with the Janeway model of microbial immunity. This latter states that recognition of nonself is at the heart of all immune responses (99).

Concluding Remarks

Innate immunity is a highly sophisticated system regulated through PRRs. It is remarkable how far the landscape of innate immunity has changed since Charles Janeway predicted the existence of PRRs in 1989 (100). The discovery of TLRs and other PRRs has also transformed our understanding of the kidney in health and disease. In this review, we have highlighted the shared functions between renal epithelial cells and professional immune cells. We discussed both the deleterious and beneficial aspects of renal epithelial TLRs. Furthermore, TLRs expressed in other nonimmune cells are also an integral component of the regional immunity. As exemplified by the recent failures of TLR4 inhibitor clinical trials, the path to tame the highly sophisticated innate immune system remains challenging. Perhaps progress is also needed in understanding and modifying the "tissue response" to the immune system. In that regard, the phenomenon of endotoxin tolerance following preconditioning might offer insights into novel mechanisms of protective adaptation. Indeed, it is now recognized that preconditioning results in tissue protection along with a preserved capacity to fight and contain infections. The mechanisms involved in endotoxin preconditioning could in turn be targeted selectively or globally to enhance tissue protection in the face of an exaggerated innate immune response (42–44,101). These are indeed exciting times for the renal research community.

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Molecules Great and Small: The Complement System

Douglas R. Mathern and Peter S. Heeger

Abstract

The complement cascade, traditionally considered an effector arm of innate immunity required for host defense against pathogens, is now recognized as a crucial pathogenic mediator of various kidney diseases. Complement components produced by the liver and circulating in the plasma undergo activation through the classical and/or mannose-binding lectin pathways to mediate anti-HLA antibody-initiated kidney transplant rejection and autoantibody-initiated GN, the latter including membranous glomerulopathy, antiglomerular basement membrane disease, and lupus nephritis. Inherited and/or acquired abnormalities of complement regulators, which requisitely limit restraint on alternative pathway complement activation, contribute to the pathogenesis of the C3 nephropathies and atypical hemolytic uremic syndrome. Increasing evidence links complement produced by endothelial cells and/or tubular cells to the pathogenesis of kidney ischemia-reperfusion injury and progressive kidney fibrosis. Data emerging since the mid-2000s additionally show that immune cells, including T cells and antigen-presenting cells, produce alternative pathway complement components during cognate interactions. The subsequent local complement activation yields production of the anaphylatoxins C3a and C5a, which bind to their respective receptors (C3aR and C5aR) on both partners to augment effector T-cell proliferation and survival, while simultaneously inhibiting regulatory T-cell induction and function. This immune cell–derived complement enhances pathogenic alloreactive T-cell immunity that results in transplant rejection and likely contributes to the pathogenesis of other T cell–mediated kidney diseases. C5a/C5aR ligations on neutrophils have additionally been shown to contribute to vascular inflammation in models of ANCA-mediated renal vasculitis. New translational immunology efforts along with the development of pharmacologic agents that block human complement components and receptors now permit testing of the intriguing concept that targeting complement in patients with an assortment of kidney diseases has the potential to abrogate disease progression and improve patient health.

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Introduction

The complement system, traditionally considered a component of innate immunity required for protection from invading pathogens, has been implicated in the pathogenesis of autoimmune kidney disease since the 1960s (1). Fifty years later, the detailed complexities of complement's role in kidney injury are still being unraveled. Building on early work indicating that macrophages and tubular cells produce complement (2,3), studies performed since the 2000s have altered former paradigms by showing that tissue-derived complement and immune cell–derived complement can each mediate local inflammation and that complement acts as a bridge between innate and adaptive immunity in an array of kidney diseases. Herein, we will review the physiology of the complement system, provide a framework for understanding complement's varied roles in kidney disease pathogenesis, and highlight potential therapeutic targets.

Biology of the Complement System

The complement system is comprised of >30 soluble and surface-expressed proteins, many of which are zymogens (inactive precursors that require cleavage to become active enzymes). In the latest nomenclature, the smaller cleavage fragments are designated as a (*e.g.*, C3a), and the larger cleavage fragments are denoted as b (*e.g.*, C3b). After they are activated, individual enzymes have the ability to repeatedly cleave their substrates, yielding a self-amplifying cascade. The various components can be considered as principally involved in (*1*) initiating complement activation, (*2*) amplifying complement activation, (*3*) performing effector functions, and/or (*4*) regulating the cascade (Figure 1).

Activation

Complement activation can be initiated through three pathways (Figure 1) (reviewed in ref. 4). The classical pathway is activated when the hexameric C1q, as part of a C1qrs complex containing two C1r molecules and two C1s molecules, binds to the Fc regions of IgG or IgM. Complement activation through the classical pathway is optimally activated by a hexameric organization of antigen-bound antibodies, a configuration that increases the avidity between C1q and the Fc regions by 20-fold (5). After an induced conformational change, the C1s component cleaves C4 to C4a+C4b and then cleaves C2 to C2a+C2b. C4b can bind to cell surfaces by a thio-ester bond, after which C2b is recruited to form the C4b2b classical pathway C3 convertase capable of cleaving C3 into C3a (an anaphylatoxin) plus C3b.

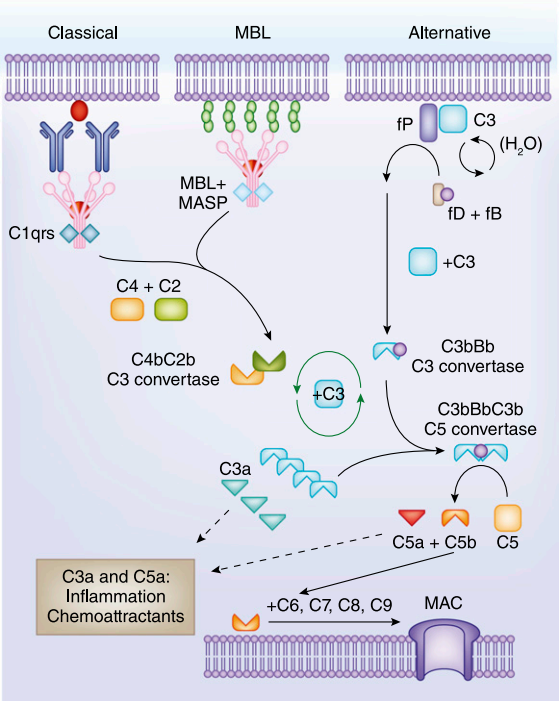

Figure 1. | **Overview of the complement cascade.** The complement cascade can be initiated by three pathways: (*1*) the classical pathway, (*2*) the mannose-binding lectin (MBL) pathway, and (*3*) the alternative pathway. The resultant C3 convertases can continuously cleave C3; however, after they are generated, the alternative pathway C3 convertase dominates in amplifying production of C3b (green looping arrow). The C3 convertases associate with an additional C3b to form the C5 convertases, which cleave C5 to C5a+C5b. C5b recruits C6, C7, C8, and 10–16 C9 molecules to generate the terminal membrane attack complex (MAC), which inserts pores into cell membranes to induce cell lysis. C3a and C5a are potent signaling molecules, which through their G protein–coupled receptors C3aR and C5aR, respectively, can promote inflammation, chemoattraction of leukocytes, vasodilation, cytokine and chemokine release, and activation of adaptive immunity. fB, factor B; fD, factor D; fP, factor P; MASP, mannose-binding lectin-associated serine protease.

In the lectin pathway, hexamers of mannose-binding lectins (MBLs) bind to bacterial carbohydrate motifs (including mannose). MBL-associated serine proteases (MASPs) function similarly to C1r and C1s to cleave C4 and then C2, generating the C4bC2b C3 convertase.

In the alternative pathway, complement activation occurs spontaneously and continuously at a low rate (referred to as tickover). The mechanism involves C3 associating with a water molecule to form C3 (H_2O), which recruits factor B (fB) and factor D (fD). fD enzymatically cleaves fB, yielding Bb, the active serine esterase that cleaves C3 to C3a+C3b. C3b associates with Bb to form the C3bBb alternative pathway C3 convertase. The thio-ester bond on C3 covalently reacts with various residues on cell surfaces, localizing C3 convertase formation predominantly to these sites. Properdin has dual functions, directly binding to microbial targets to provide a platform for assembly of the alternative pathway C3 convertase (6) and increasing the stability of the C3bB/C3bBb complexes (7).

Amplification

The C3 convertases repeatedly cleave C3 molecules, yielding multiple C3b products, each of which can interact with fB to form more C3 convertases. As consequences, C3 cleavage is the central amplification step of the cascade, and regardless of the initial activation pathway, amplification at the C3 convertase step occurs through the alternative pathway. Regulation of the C3 convertase amplification step is crucial to restrain complement activation so as to prevent pathologic consequences (see below).

Effector Functions

C4b2b and C3bBb form multimeric complexes with additional C3b molecules, yielding the C5 convertases C4b2bC3b and C3bBbC3b. These enzymes cleave C5 to C5a (an anaphylatoxin) plus C5b, the latter of which binds to C6 and subsequently facilitates binding of C7 and C8 plus 10–16 C9 molecules to form the C5b-9 membrane attack complex (MAC) (Figure 1). The MAC forms a pore in cell membranes, which promotes lysis of non-nucleated cells (*e.g.*, bacteria and human red blood cells [RBCs]). Insertion of MACs into nucleated host cells generally does not result in lysis but can induce cellular activation (8) and/or promote tissue injury (9).

Various complement cleavage products have other effector functions (Figure 2, Table 1). C3a and C5a ligate their seven transmembrane-spanning G protein–coupled receptors C3aR and C5aR, respectively, transmitting proinflammatory signals that induce vasodilation and cytokine and chemokine release. They also mediate neutrophil and macrophage chemoattraction, activate macrophages to promote intracellular killing of engulfed organisms, and contribute to T-cell and antigen-presenting cell (APC) activation, expansion, and survival (see below) (10–13). C3b and other bound cleavage products bind to various surface-expressed receptors, including complement receptor 1 (CR1), CR2, CR3, and CR4, functioning as opsonins.

Regulation

Complement activation must be physiologically restrained to limit damage to self-cells (4). Complement regulation occurs at multiple steps through distinct mechanisms (Figure 3). Regulation of C3 convertase activity is accomplished by multiple molecules with overlapping but discrete functions. Decay accelerating factor (DAF; CD55) is a glycophosphatidylinositol (GPI)-anchored, membrane-bound regulator that accelerates the decay of cell surface–assembled classical and alternative pathway C3 and C5 convertases (facilitating disassociation of Bb from C3bBb and C2b from C4b, while also competitively inhibiting their reformation) (14), thereby preventing amplification, downstream cleavage events, and formation of the MAC (15). This decay accelerating activity functions *intrinsically* (*i.e.*, restraining complement activation only on the cell surface on which DAF is expressed).

Membrane Cofactor Protein (MCP; CD46) and its murine homolog *Crry* are surfaced-expressed regulators with cofactor activity (16) functioning as cofactors for serum factor I (fI), which cleaves C3b to iC3b, thereby irreversibly preventing reassembly of the C3 convertase. *Crry* also exhibits decay accelerating activity (17). The cleavage product iC3b (an opsonin) can be further broken down to C3c and C3dg

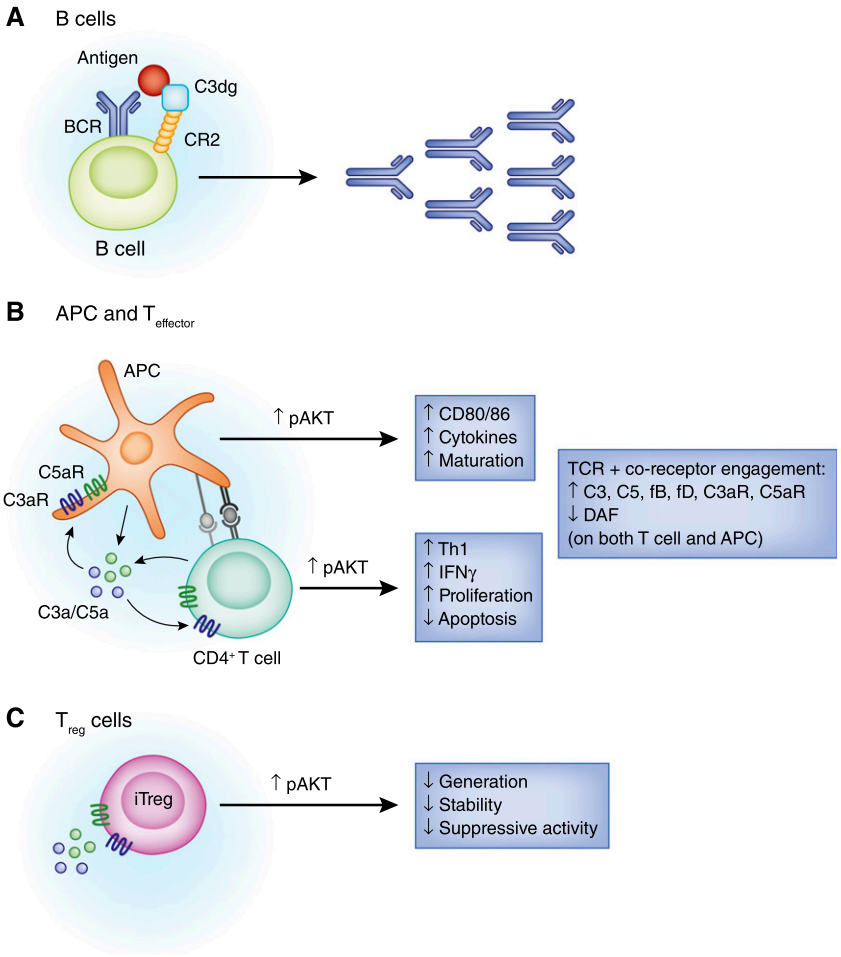

Figure 2. | **Complement and adaptive immunity.** (A) B cells express complement receptor 2 (CR2; CD21), which binds to C3dg, a C3 breakdown product that functions as an opsonin. C3dg-coated antigens recognized by antigen-specific B-cell receptors plus CR2 initiate engulfment and lower the threshold of B-cell activation, promoting antibody production. (B) Cognate interactions between T cells and antigen-presenting cells (APCs) (T-cell receptor [TCR] +CD28/80/86 or CD40/154) upregulate the expression of multiple alternative pathway components, including C3, C5, fB, fD, C3aR, and C5aR, while simultaneously downregulating decay accelerating factor (DAF) expression (lifting restraint on complement activation). Locally produced C3a and C5a act in an autocrine/paracrine manner through AKT signaling to promote maturation and cytokine production of APCs, Th1/IFN-γ expression, increased proliferation, and decreased apoptosis of T cells. (C) Regulatory T-cell generation, stability, and suppressive function are decreased by C3a and C5a signaling-induced AKT signaling, which impairs nuclear translocation of Foxo1, a transcription factor for FoxP3. AKT, phosphokinase B; pAKT, phosphorylated phosphokinase B; BCR, B cell receptor; iTreg, murine-induced regulatory T cell.

(through fI- and cofactor-dependent cleavage processes) (reviewed in ref. 18), the latter of which interacts with CR2 on B cells to facilitate B-cell activation (19).

Factor H (fH) is a plasma protein that also regulates complement activation at the C3 convertase step (reviewed in ref. 20). The carboxy terminus of this protein binds surface-deposited C3b and surface-expressed polyanionic glycosaminoglycans, including sialic acid residues. After they are bound, the N-terminal domains of fH exhibit decay accelerating and cofactor activities (Figure 3). fH restrains complement activation on host surfaces that do not express other complement regulators, including exposed basement membranes in the glomerulus (which express glycosaminoglycans), explaining, in part, the association between mutations in fH or fI and various C3 nephropathies (see below).

Additional complement regulators (Figure 3) include the GPI-anchored and surfaced-expressed protein protectin (CD59), which blocks formation of the MAC, the surface-expressed CR1, which exhibits decay accelerating activity and cofactor activity for fI, and C1 inhibitor, a serine protease that irreversibly binds to and inactivates C1r, C1s, MASP-1, and MASP-2, thereby limiting classical and MBL pathway activation. Ubiquitously expressed carboxypeptidases rapidly inactivate the anaphylatoxins C3a and C5a (reviewed in ref. 4).

Sources of Complement

Liver-derived plasma complement is essential for protection from pathogens and contributes to antibody-initiated, complement-mediated autoimmune injury. Complement components can be produced by tissue-resident (*e.g.*, tubular cells in the kidney [21]) and migratory/immune cells, including T cells and APCs (22–24). A thorough understanding of complement-mediated kidney disease requires consideration of the source of complement production, the site of complement activation, the specific complement

Table 1. Complement receptor functions

Complement Receptor	Alternative Names	Ligand	Effector Functions	Cell Type
CR1	CD35, immune adherence receptor	C3b, iC3b, C4b, C1q	Clearance of immune complexes, enhancement of phagocytosis, and regulation of C3 breakdown	Many nucleated cells and RBCs, B cells, leukocytes, monocytes, and follicular dendritic cells
CR2	CD21, Epstein–Barr virus receptor	C3dg, C3d, iC3b	Regulation of B-cell function, B-cell coreceptor, and retention of C3d-tagged immune complexes	B and T cells and follicular dendritic cells
CR3	MAC1, CD11b-CD18, $\alpha M\beta 2$ integrin	iC3b, factor H	iC3b enhances contact of opsonized targets, resulting in phagocytosis	Monocytes, macrophages, neutrophils, NK cells, eosinophils, myeloid cells, follicular dendritic cells, and $CD4^+$ and $CD8^+$ T cells
CR4	CD11c-CD18, $\alpha X\beta 2$ integrin	iC3b	iC3b-mediated phagocytosis	Monocytes and macrophages

Modified from reference 130, with permission. CR1, complement receptor 1; RBC, red blood cell; MAC1, membrane attack complex 1.

effector components and receptors involved, and the function of complement regulators in each situation.

Links between Complement and Adaptive Immunity

It has been known for decades that complement depletion impairs antibody production (25). The mechanism involves antigen-bound C3dg (an iC3b cleavage product) binding to B cell–expressed CR2 (CD21), which facilitates antigen presentation to B cells and lowers the threshold for B-cell activation (26) (Figure 2).

Work published since the early 2000s uncovered an unexpected role for complement as a regulator of T-cell immunity. During cognate interactions between T cells and APCs, both partners upregulate and secrete alternative pathway complement components C3, fB, and fD, produce C5, and upregulate surface expression of C3aR and C5aR (23,24) (Figure 2). These changes are a consequence of costimulatory molecule signaling by CD28/CD80/CD86 and CD154/CD40 (24), which simultaneously and transiently reduces cell surface–expressed DAF (thereby lifting restraint on complement activation). Locally produced C3a and C5a bind to their receptors and function as autocrine and paracrine stimulators of the T cell and the APC (23,24). Signaling through these GPCRs in T cells activates phosphoinositide-3-kinase-γ and induces phosphorylation of phosphokinase B (AKT) (22,24), upregulating the antiapoptotic protein Bcl-2 and downregulating the proapoptotic molecule Fas. Together, these complement-dependent mechanisms enhance T-cell proliferation and diminish T-cell apoptosis (22). C3aR/C5aR signaling is also required for T-cell homeostasis, because T cells deficient in both receptors spontaneously undergo accelerated cell death *in vitro* and *in vivo* (24). The observations derived from murine models also apply to human T cells (27). Building on these findings, a 2013 publication showed that resting human $CD4^+$ T cells contain C3 in granules that is rapidly cleaved by cathepsin-L to C3a and secreted after CD3 ligation. Evidence suggests that this intracellular C3/C3a contributes to the aforementioned promotion of T-cell survival and effector responses (28).

Regulatory T cells (Tregs) are instrumental for allograft tolerance induction and maintenance in rodents and associated with improved long-term transplant outcomes in humans (29). Data published in 2013 indicate that complement also regulates Treg induction, function, and stability (12,30) (Figure 2). Our group showed that peripheral, murine, natural regulatory T cells (nTregs) express C3aR and C5aR and that signaling through these receptors inhibits Treg function (11). Genetic and pharmacologic blockade of C3aR/C5aR signal transduction in nTreg cells augments their *in vitro* and *in vivo* suppressive activity. Mechanisms involve C3a/C5a-induced phosphorylation of AKT and, as a consequence, phosphorylation of the transcription factor Foxo1, which results in lowered nTreg Foxp3 expression. Two additional sets of data showed that genetic deficiency or pharmacologic blockade of C3aR/C5aR signaling augments murine-induced regulatory T cell (iTreg) generation, stabilizes Foxp3 expression, and resists iTreg conversion to IFN-γ/TNF-α–producing effector T cells (12,30). Pharmacologic antagonists to human C3aR and C5aR also augment *in vitro* generation and stability of human iTreg from naïve precursors (12,30). These new results build on previously published evidence that coengagement of the T-cell receptor

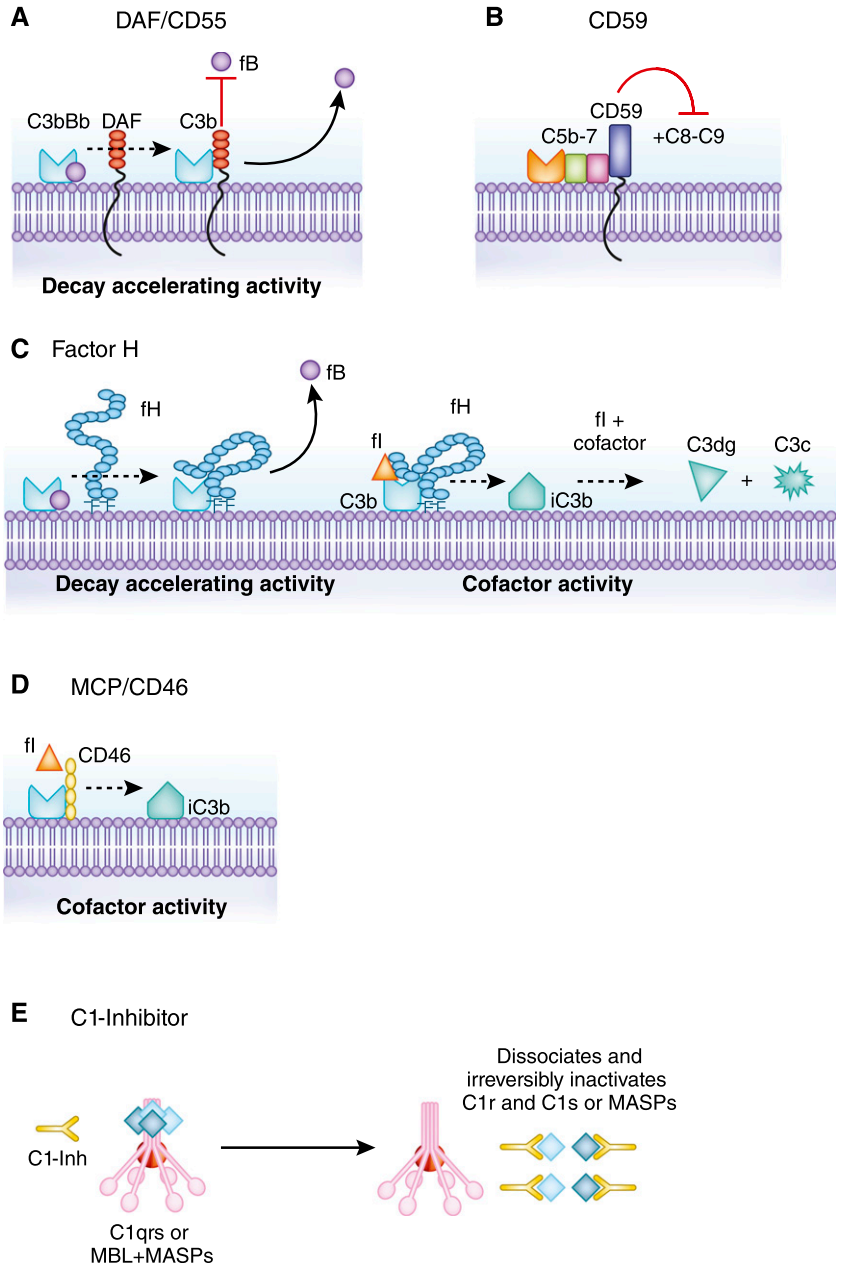

Figure 3. | **Complement regulation.** (A–D) Schematics depicting decay accelerating activity of (A and C) decay accelerating factor (DAF)/CD55 and factor H (fH), (B) CD59, which inhibits membrane attack complex formation, and (D) Membrane Cofactor Protein (MCP)/CD46 and fH, which display cofactor activity for factor I (fI). Cofactor-mediated fI activity irreversibly cleaves C3b to iC3b and subsequently cleaves iC3b to C3c and C3dg. (E) Schematic depicting the mechanism of C1-inhibitor (C1-inh), a protease that inactivates C1r, C1s, and mannose-binding lectin-associated serine proteases (MASPs), irreversibly preventing reformation of the classical and mannose-binding lectin (MBL) pathways initiating complexes. C1-inh also inhibits the kallikrein-kinin and coagulation cascades, two other mechanisms of complement activation.

and the complement regulator CD46 promotes regulatory IL-10 production (31) to delineate a crucial role for complement in modulating the balance between pathogenic and protective adaptive T-cell responses.

Complement and Kidney Disease

Antibody-Initiated Activation of Serum Complement

Autoantibodies reactive to kidney-expressed self-antigens and/or antibody/antigen complexes deposited in the kidney are considered causative of various human kidney diseases. Increasingly available evidence links the pathogenesis of many of these antibody-initiated kidney pathologies to complement-derived effector mechanisms, in which plasma complement is activated through the classical or MBL pathways (Figure 4).

Membranous Nephropathy. Membranous nephropathy (MN), a common cause of nephrotic syndrome in adults, is characterized by a fine granular deposit of IgG with C3 in the peripheral capillary loops (32,33). IgG4 reactive to the

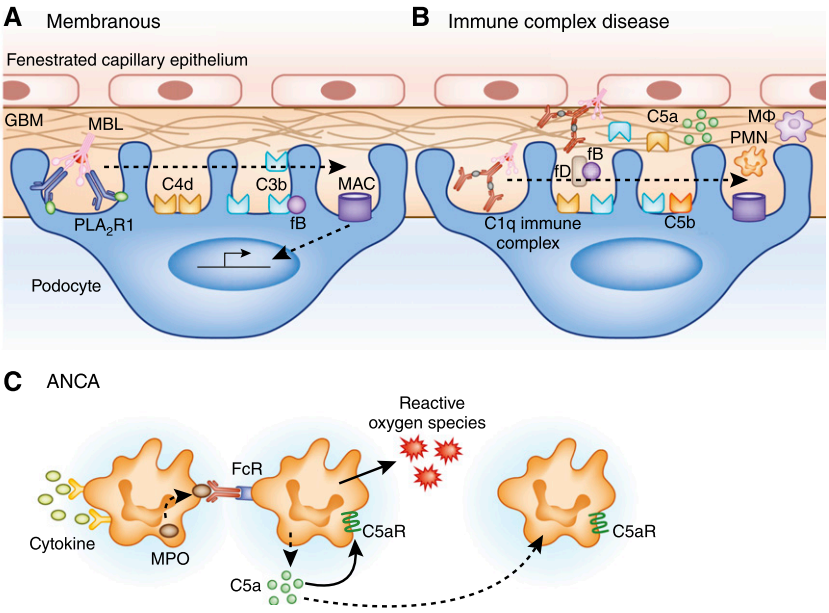

Figure 4. | Mechanisms through which complement mediates antibody-initiated kidney diseases. (A) Complement and membranous nephropathy (MN): autoantibodies reactive to podocyte-expressed phospholipase A_2 receptor 1 (PLA_2R1) activate complement through the mannose-binding lectin (MBL) pathway. Cascade amplification promotes membrane attack complex (MAC) formation, which induces sublytic signaling and dedifferentiation of the podocyte to impair its filtration capacity, leading to proteinuria. (B) Complement and immune complex disease: circulating or *in situ*–formed immune complexes from kidney disease initiated by lupus, streptococcal infections, and cryoglobulinemia are deposited in the subepithelial and/or subendothelial space of the glomerulus. Classical pathway activation induces inflammation and recruitment of neutrophils and macrophages (MΦ), promoting tissue injury. (C) Complement and ANCA vasculitis. Cytokine-induced neutrophil activation results in surface expression of myeloperoxidase (MPO; among other cytoplasmic antigens). On binding to ANCA and cross-linking FcR, local complement activation results in C5a production, which induces vascular inflammation by ligating its receptor C5aR. GBM, glomerular basement membrane; PMN, polymorphonuclear leukocyte.

M-type phospholipase A_2 receptor, a transmembrane glycoprotein expressed on the glomerular podocyte, is present in 70%–98% of patients with MN (34,35). Although IgG4 does not efficiently activate complement through the classical pathway, deposition of C4d, a breakdown product of C4b, is detectable in essentially 100% of patients with primary MN (36,37). Together with the observations that MBL and hypogalactosylated IgG (including IgG4) can be detected in subepithelial deposits in primary MN and that hypogalactosylated IgG can bind to MBL and activate complement through the MBL pathway, the data suggest that MBL-initiated complement activation is pathogenic in MN. MACs are detectable in the urine of patients with MN and considered a dynamic marker of ongoing injury, supporting an integral role for complement in MN (reviewed in ref. 38).

Mechanistic studies in animal models, including Heymann Nephritis, indicate that antipodocyte antibodies lead to insertion of MAC into podocytes and that blocking MAC formation prevents phenotypic expression of disease (39). The resultant sublytic podocyte activation alters cytoskeletal structure crucial for foot process and slit diaphragm integrity and function, leading to proteinuria (40,41). Associated promotion of extracellular matrix results in the characteristic thickened glomerular basement membranes (GBMs) observed in this disease (42).

Although one report in abstract form (G. Appel *et al.*, unpublished data) suggested that anti-C5 mAb had no effect on proteinuria in patients with MN, additional studies are needed to determine if targeting complement is an effective therapy for this disease.

Anti-GBM Disease. Autoantibodies targeting the NC1 domain of type IV collagen are pathogenic mediators of anti-GBM disease (43). The proliferative GN observed in anti-GBM disease is characterized by linear deposition of IgG and various complement components along the GBM (44). The classical and alternative pathways are implicated, because MBL, C1q, fB, properdin, C3d/C4d, and C5b-9 have been detected in GBM. An observed correlation between intensity of fB deposition and glomerular crescent formation supports a pathogenic link (45). Evidence indicates that local complement activation results in C3a- and C5a-mediated inflammation as well as MAC-dependent sublytic activation of cells within the glomerulus, which together promote nephropathy and extracellular matrix formation (46). Together, these mechanistic findings support the need to test whether complement inhibition positively affects outcomes in patients with anti-GBM disease.

Immune Complex-Initiated Glomerular Diseases. Circulating immune complexes that are deposited in the subepithelial or subendothelial compartments of the glomerulus can also mediate complement-dependent glomerular injury, including GN associated with streptococcal infection, cryoglobulinemia, and lupus. These disease processes are commonly characterized by neutrophils and C3 deposits in glomeruli and systemic C3 depletion, suggestive of a

pathogenic role of complement-mediated inflammation and immune cell recruitment (47).

Animal model data supporting complement activation as pathogenic in lupus nephritis include the observation that fH-deficient MRL/lpr mice died with severe, diffuse GN (48). Conversely, administration of a CR2-*Crry* fusion protein that targets complement regulation to C3b deposits (CR2 binds C3b) prevented expression of disease (49). In MRL/lpr mice, C5aR blockade decreased glomerular inflammation (50). Anti-C5 mAb ameliorated GN in the murine NZB/W(F1) lupus model (51), indicating a role for terminal complement. A phase 1 human trial with eculizumab (anti-C5) suggested preliminary efficacy, but the treatment period was too brief to draw definitive conclusions (52). Despite these observations, complement is not the sole pathogenic mediator of lupus nephritis, because FcR deficiency but not C3 deficiency prevented phenotypic expression of disease in one model (53).

The complexities of complement's effects on lupus are illustrated by the seemingly paradoxical observation that genetic absence of C4 or C1q in mice (54) and humans (55) increases the risk of developing lupus nephritis. The mechanism is likely related to an absence of complement-derived opsonins, preventing clearance of immune complexes that deposit in the glomerulus and promote FcR-dependent inflammation.

ANCA-Induced Vasculitis. ANCAs contribute to small vessel vasculitis, which is characterized by a paucity of Ig deposits (56) but with complement component deposition (Bb, C3d, C3c, and C5b-9) at sites of acute vascular and glomerular inflammation (57). Cytokine-primed neutrophils display ANCA-binding antigens (myeloperoxidase [MPO] and proteinase 3) on their surfaces and participate in vascular injury. Complement depletion protected anti–MPO-treated mice from developing necrotizing crescentic GN (58). fB deficiency was protective, but C4 deficiency was not protective, implicating the alternative pathway. Whereas C5 deficiency or blocking anti-C5 mAb was protective (59), C6 deficiency was not protective (60), indicating that C5 cleavage but not MAC formation is pathogenic. Additional animal studies showed that ANCAs stimulate neutrophils to produce and release C5, and blockade or deficiency of C5aR (60) prevented disease expression. Together, the data suggest that pathology is mediated by ANCA-induced, neutrophil-derived complement release and leads to C5a/C5aR-induced proinflammatory signaling, particularly in neutrophils and neutrophil-associated vasculature (61), rather than by MAC formation. A small molecule C5aR inhibitor limited expression of a murine model of anti–MPO-induced kidney disease (60), and C5aR antagonism is being tested as a therapy for patients with ANCA vasculitis (European Union Clinical Trials Register ID EUCTR2011–001222015-GB).

Other Glomerular Diseases. IgA nephropathy, characterized by recurrent bouts of GN, focal mesangial cell expansion, and IgA deposition, is likely mediated by MBL-dependent and/or alternative pathway-dependent, antibody-initiated complement activation (62). Deposits of C3 and C5b-9 are detectable in the diseased glomeruli and correlate with disease severity and prognosis. Experimental evidence suggests that sublytic MAC activates mesangial cells, yielding mesangial proliferation and matrix expansion (62).

The pathogenesis of FSGS remains unclear, but IgM and C3 deposits are commonly observed in the affected glomeruli (63). Mutations in fH and C3 have been described in cases of FSGS (64), and a murine model of IgG-initiated FSGS in DAF-deficient mice (65) supports a role for complement dysregulation in some cases. Complement inhibition has not been carefully studied as a therapy for FSGS.

Postinfection GN, classically after *Streptococcal pyogenes* infection, is characterized by proliferative GN and deposition of C3 with or without Igs (reviewed in ref. 66). Although the majority of patients achieve complete remission of the associated nephritic syndrome, some experience delayed resolution or chronic GN, resulting in ESRD. A recent study published on 11 patients at the Mayo Clinic found multiple underlying causes of alternative pathway dysregulation in these chronic patients, including mutations in fH or CFHR5 and/or the presence of C3 nephritic factors (67).

Monoclonal gammopathy has also been associated with the activation of the alternative pathway and subsequent induction of membranoproliferative GN. Circulating λ–light-chain dimers were found to bind to fH and inhibit its control function, thus lifting restraint over alternative pathway regulation, but they differed from C3 nephritic factor in that the dimers were unable to bind and stabilize the alternative pathway C3 convertase (68).

Antibody-Initiated Injury to a Kidney Transplant. Donor-reactive anti-HLA antibodies are considered pathogenic mediators of acute and chronic transplant injury (69). They can bind to donor tissue and mediate damage through multiple mechanisms, including complement activation (70,71).

A mechanistic link between antibody-mediated injury and terminal complement activation was documented through experiments performed in rodents: whereas heart allografts transplanted into sensitized (with preexisting antidonor antibodies) wild-type rats were rejected in 6–7 days, graft survival was prolonged to >30 days in sensitized $C6^{-/-}$ recipients (72). Documentation of impaired MAC complex (C5b-9) formation in the $C6^{-/-}$ recipients (73) supports the conclusion that terminal complement is a key effector mechanism. In work by others, anti-C5 mAb (inhibits C5 cleavage) plus cyclosporin and short-term cyclophosphamide resulted in prolonged heart allograft survival in presensitized mice, despite persistent antidonor IgG in the sera and the graft (74).

In 2013, studies in a humanized mouse model (8) showed that antidonor HLA antibodies bind to human aortic endothelium to initiate complement activation, resulting in MAC insertion into aortic endothelial cells. This induced endothelial cell activation, characterized by noncanonical NF-κB activation and upregulated production of chemokines, cytokines, and adhesion molecule expression, which in turn, facilitated T cell–mediated injury to the aortic allograft (8). The findings support the conclusion that complement bridges pathogenic humoral and cellular alloimmunity to mediate tissue damage.

Clinical studies have begun to test the efficacy of complement-targeted strategies to treat human antibody-mediated transplant rejection. Anti-human C5 mAb plus plasma exchange reduced the incidence of antibody-mediated rejection in 26 sensitized recipients of kidney transplants (75) and successfully reversed established antibody-mediated rejection in a small cohort (76).

Complement-Based Diagnostics Relevant to Transplantation. The recognition that complement participates in antibody-initiated allograft rejection suggested that identifying serum anti-HLA antibodies capable of binding C1q would enhance their prognostic use after kidney transplantation (77). A 2013 paper, indeed, suggests that, among patients with serum anti-HLA antibodies, those binding to C1q+ had the worst kidney graft survival (78). In another example of complement-based diagnostics, C4d staining of kidney transplant tissue is currently considered one key criterion for diagnosing antibody-mediated allograft rejection (79).

Kidney Injury Mediated by Serum Complement in the Absence of Antibody

Emerging evidence suggests that unrestrained C3 convertase activity underlies the pathogenesis of several diseases involving the kidney, including paroxysmal nocturnal hemoglobinuria (PNH), forms of C3 nephropathy, and atypical hemolytic uremic syndrome (aHUS) (Figure 5).

PNH. PNH is a hematologic disorder characterized by bone marrow failure, thrombophilia, complement-mediated intravascular hemolysis, and hemoglobinuria. It is caused by a clonal expansion of RBC precursors that contain a mutation in the X-linked phosphatidylinositol glycan anchor biosynthesis, class A gene (PIGA) that encodes for a protein involved in GPI anchor synthesis (80). DAF and CD59 are GPI-anchored molecules that require posttranslational addition of GPI anchors to guide them to cell surfaces. The PIGA mutation prevents DAF and CD59 surface expression on the affected RBCs. The inability to regulate alternative complement activation/amplification at the C3 convertase step (absent DAF) and prevent MAC formation (absent CD59) causes spontaneous lysis of the mutant RBCs. Therapeutically inhibiting MAC formation with an anti-C5 antibody that prevents C5 cleavage reduces morbidity and increases quality of life for patients with PNH (81). Although effective in preventing lysis, the anti-C5 mAb does not affect the unregulated upstream production and deposition of C3b on RBC surfaces resulting from the absence of DAF. Evidence indicates that this C3b deposition functions as an opsonin, causing macrophage-dependent RBC destruction in the liver/spleen, despite anti-C5 therapy (82). Alternative treatment strategies, including those targeting C3 activation (83), require additional study.

C3 Nephropathies. C3 nephropathies are nephritic kidney diseases characterized by low serum C3 and glomerular C3 deposits without IgG. Subsets of C3 nephropathies are associated with serum C3 nephritic factors (Figure 5): acquired autoantibodies that impair complement regulation by binding directly to the C3bBb C3 convertase or its components, enhancing properdin-mediated stabilization of the complex, and/or inhibiting fH-mediated C3b degradation (84).

C3 nephropathies can also occur in association with genetic mutations in complement components and/or regulators that result in impaired complement regulation (Figure 5) (84). Loss-of-function mutations in fH, gain-of-function mutations in C3 (conferring resistance to fH-mediated cleavage), and gain-of-function mutations in complement fH regulatory proteins (which compete with fH for binding to C3b and impair the function of fH) have been described (84).

Regardless of the specific molecular basis for the complement dysregulation, the resultant complement activation likely predominantly causes glomerular disease, because the glomerulus contains fenestrated endothelium with exposed GBM that requires functional fH and fI to prevent local complement activation. Supporting this concept, fH-deficient mice develop membranoproliferative GN with low serum C3 levels (85), and recombinant fH restores plasma C3 levels with resolution of C3 deposition in the GBM (86). Mice deficient in both fH and fB do not develop disease, confirming a pathogenic role for alternative pathway activation (85). $fH^{-/-}/C5^{-/-}$ mice and $fH^{-/-}$ mice treated with anti-C5 mAb developed less severe disease, whereas C6-deficient mice were not protected, inferring a role for C5a/C5aR in immune cell recruitment (87).

Effective therapy for C3 nephropathies remains enigmatic, but limited studies in animal models and patients have suggested that restoration of alternative pathway regulation may prove effective. Infusions of fresh frozen plasma containing fH may benefit patients deficient in fH (88), and therapy targeting C5 showed some success in patients with forms of C3 nephropathies (89,90).

aHUS. The current concept is that aHUS, characterized by hemolysis and renal failure associated with kidney endothelial cell injury, typically in the absence of detectable complement deposition in the glomerulus (91), is also a result of complement dysregulation. Inherited loss-of-function mutations in fI, MCP, and fH as well as acquired, blocking, anti-fH antibodies have been associated with cases of aHUS (92). Gain-of-function mutations in C3 and/or fB, which promote accumulation of the C3bBb convertase and overwhelm/resist complement regulation, have been described (93).

Additional insights derived from work using mice with a mutated fH were that they lack the ability to bind to cell surfaces but maintain its complement-regulatory capacity (Figure 5). Although complement activation in the plasma was controlled, the animals developed C5-dependent features of thrombotic microangiopathy (94,95), indicating that fH must regulate complement activation while bound to surfaces in the kidney to prevent disease.

Most humans with inherited mutations associated with aHUS or C3 nephropathy are heterozygotes. Current concepts are that common allelic variants in complement regulators present in the general population confer a complotype that predisposes to disease (96) and that additional immune insults unmask complement regulatory deficiencies. As one illustration supporting this concept, poststreptococcal GN, generally a self-limited disease, resulted in progressive C3 nephropathy in a patient with a complement regulator mutation (97).

Control of complement activation using eculizumab (anti-C5) has revolutionized treatment of aHUS. Approximately 85% of treated patients have achieved remission (98). Those refractory to eculizumab may have disease driven by C3 cleavage products (which would not be affected by anti-C5 mAb), raising the possibility that targeting C3 and/or C3a/C3aR may ultimately prove to be more effective.

Kidney-Derived Complement and Disease

Ischemia-Reperfusion Injury. Ischemia-reperfusion (IR) injury results from tissue hypoxia, mitochondrial damage,

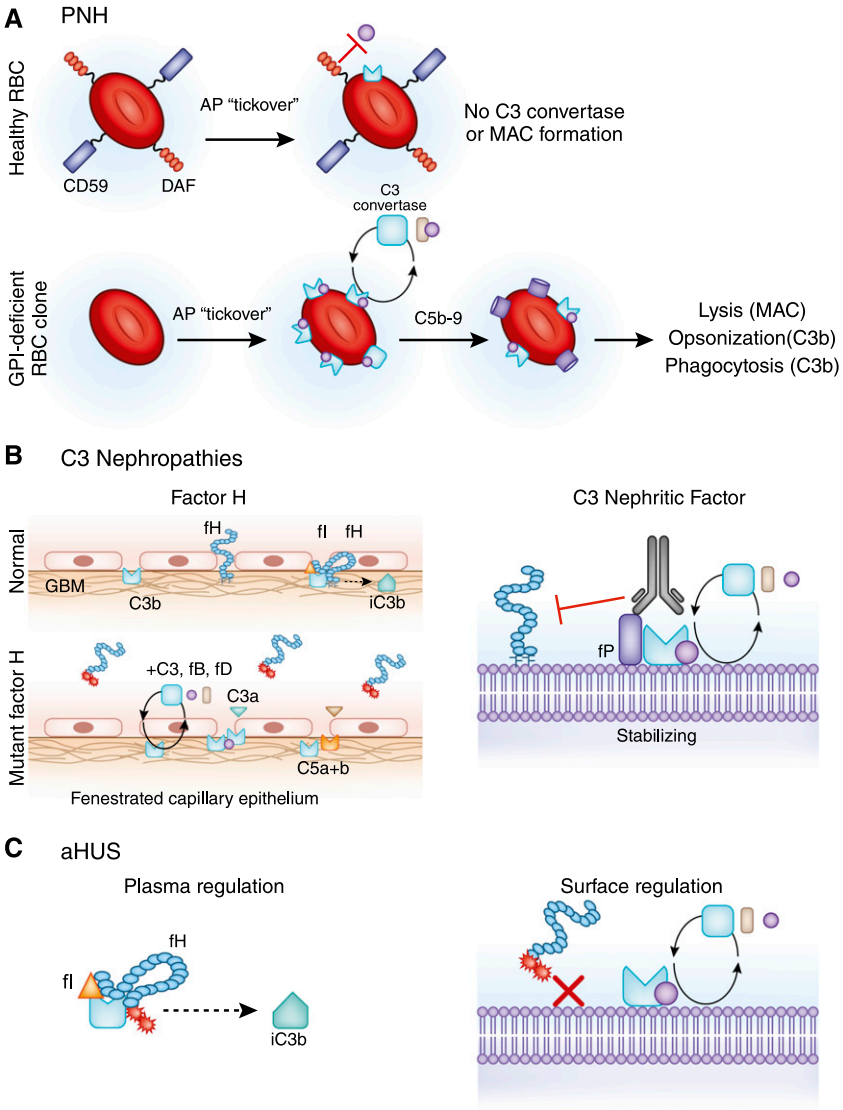

Figure 5. | **Kidney diseases caused by abnormalities in complement regulation.** (A, upper panel) Healthy red blood cells (RBCs) express CD59 and decay accelerating factor (DAF), preventing complement activation on their surfaces. (A, lower panel) Paroxysmal nocturnal hemoglobinuria (PNH) is caused by mutation of the X-linked phosphatidylinositol glycan anchor biosynthesis, class A gene, resulting in a loss of glycophosphatidylinositol (GPI) anchor biosynthesis that prevents surface expression of GPI-anchored DAF (CD55) and CD59. An inability to regulate alternative pathway activation/amplification at the C3 convertase step (DAF; amplification loop) and prevent MAC formation (CD59), results in spontaneous RBC lysis (membrane attack complex [MAC]). (B) C3 nephropathies. Factor H (fH) normally binds to polyanionic glycosaminoglycans on glomerular basement membranes (GBMs) to restrain complement activation (Normal). Mutations in the C-terminal region of fH (depicted as red regions of the protein) impair fH's physiologic ability to bind to the basement membrane, lifting restraint on local complement activation and contributing to glomerular inflammation and injury (mutant fH). C3 nephritic factors (right panel) can promote stability of C3, and properdin (factor P [fP]) can stabilize the C3bBb C3 convertase or inhibit fH-mediated decay/degradation, lifting restraint on C3 convertase-mediated amplification (looping arrow). (C) Atypical hemolytic uremic syndrome (aHUS). Among several mechanisms, mutations in fH that impair its ability to bind to basement membranes (red regions of protein) permit complement regulation in the fluid phase (plasma regulation; left panel) but prevent local complement regulation on surfaces (right panel), predisposing to vascular inflammation and hemolysis.

and ATP depletion followed by the generation of free oxygen radicals on reperfusion, which initially damage endothelium (99). Ensuing inflammation is driven by Toll-like receptor signaling, and cytokines, chemokines, and complement amplify the inflammation, resulting in tubular injury and kidney dysfunction (Figure 6).

To summarize findings reviewed elsewhere (100), complement deposition and loss of membrane-bound complement regulators occur during murine kidney IR injury, and overexpression of *Crry* (murine homolog of MCP) ameliorated IR injury. IR injury was dampened in complement-depleted mice and C3-deficient, fB-deficient, or C5-deficient mice. Conversely, DAF-, *Crry*-, or fH-deficient mice are more susceptible to IR injury. Reciprocal transplant studies showed that donor kidney–derived C3 and not systemic recipient C3 is the predominant mediator of IR injury (101). Using C3aR-, C5aR-, or C3aR/C5aR-deficient mice (102), investigators observed that deficiency of either or

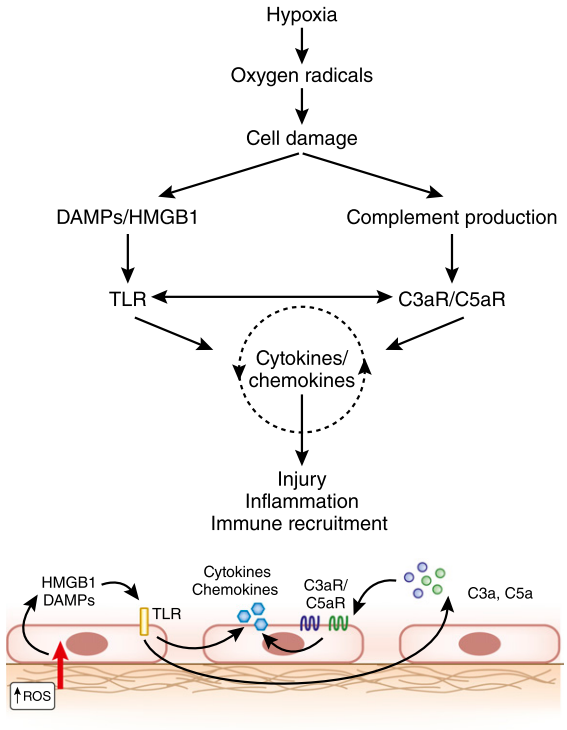

Figure 6. | **Complement in ischemia-reperfusion (IR) injury.** IR injury results from tissue hypoxia and reperfusion, promoting reactive oxygen species (ROS) production, which causes endothelial injury. Inflammation drives Toll-like receptor (TLR)–mediated signaling and cytokine production as well as complement production and activation, which drives additional cytokine and chemokine production and immune cell recruitment through C3aR and C5aR signaling. DAMP, damage associated molecular pattern; HMGB1, high mobility group protein B1.

both of these receptors protected mice from kidney IR injury and that their expression on either renal tubular epithelial cells or circulating leukocytes contributes to the pathogenesis. Together, the data indicate that IR injury upregulates production of complement components by kidney endothelial and tubular cells and infiltrating immune cells. Local activation through the alternative pathway yields C3a/C5a, which amplifies local inflammation through autocrine/paracrine ligations with their kidney cell–expressed receptors (102). Confirmatory evidence in humans includes detection of soluble C5b-9 after reperfusion of deceased donor but not living donor kidneys (103) and higher expression of complement genes in deceased versus living donor kidneys on reperfusion (104).

An analog of the human complement-regulatory protein CD35 (CR1; blocks C3 convertase) was conjugated to a myristoylated peptidyl tail, such that, when administered by intravenous perfusion of the harvested organ ex vivo, it will self-insert into the lipid bilayer of the endothelial cell membranes. This reagent was effective in preventing post-transplant kidney IR injury in rats (105). The human reagent, mirococept (APT070), is being studied in a clinical trial for prevention of DGF (100). Eculizumab is also being tested for efficacy in preventing post-transplant DGF (NCT01403389 and NCT01919346).

Chronic Kidney Injury and Fibrosis. Mechanisms of kidney fibrosis, including late kidney transplant failure, involve immune and nonimmune mechanisms (106). The functional and structural changes of chronic renal allograft failure share similarities with those observed in other forms of chronic progressive kidney disease, in which decline of functioning nephron mass has been considered the key event (107). Emerging evidence suggests that intragraft complement activation contributes to this progressive kidney injury (108). C3 is implicated in the activation of the renin-angiotensin system and the epithelial-to-mesenchymal transition (109,110). Together with observations that absence/blockade of C5/C5aR (but not blocking MAC formation) limited kidney fibrosis in several animal models (111,112), the data suggest that kidney-derived complement participates in fibrosis of native and transplanted kidneys (Figure 7).

Associative evidence linking complement to progressive human kidney transplant injury derives from studies of complement gene polymorphisms and transplant outcomes. Specific C5 polymorphisms in both the donor and recipient have been associated with worse late graft function but not risk of acute rejection (113). Although controversial, donor kidney expression of a specific polymorphic variant of C3 is associated with worse post-transplant outcomes (114,115). Additionally, proteomic studies of kidney allograft tissue revealed strong associations between chronic injury and alternative pathway but not classical pathway complement components (116). An ongoing study of chronic anti-C5 mAb therapy in kidney transplant recipients (NCT01327573) could potentially provide additional insight into the role of complement as a mediator of progressive graft dysfunction and interstitial fibrosis and tubular atrophy.

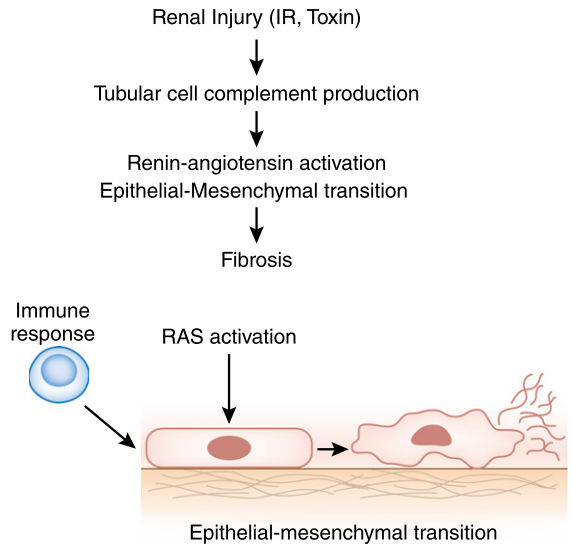

Figure 7. | **Chronic kidney injury and fibrosis.** Chronic injury caused by toxins or transplant-related immune responses upregulates complement production and activation within the kidney. C3 has been implicated in the activation of the renin-angiotensin system and the promotion of the epithelial-to-mesenchymal transition, including fibrosis. IR, ischemia reperfusion; RAS, renin-angiotensin system.

Complement and T Cell–Mediated Transplant Rejection

Extending from the fundamental discoveries that absence of donor C3 prevents murine kidney transplant rejection (117) and that immune cell–derived complement augments effector T-cell differentiation and survival (23,24), studies performed in transplant models revealed that wild-type mice reject DAF-deficient heart allografts with accelerated kinetics (118). The accelerated rejection is caused by a complement-dependent augmentation of antidonor T-cell immunity. Donor or recipient DAF deficiency accelerated skin graft rejection (23), bypassed the requirement for CD4 help in murine heart transplant rejection (119), and overcame immune privilege of the eye to cause rapid corneal transplant rejection (120). Local complement production and C5a/C5aR interactions also influence effector $CD8^+$ T-cell responses to allogeneic vascular endothelial cells (121) in *in vitro* culture systems and *in vivo* in response to a heart transplant (8,121) as well as T cell–dependent kidney transplant rejection in rodents (122). Together with the findings that anti-C5 mAb synergizes with CTLA4–Ig to prevent T-cell priming, limits T-cell trafficking to an allograft, and prolongs transplant survival in mice (123), the work supports the conclusion that complement is a physiologic regulator of pathogenic T-cell immunity that causes allograft rejection (as well as various autoimmune diseases) in animal models.

Confirmatory human experiments published in 2013 show that C3a and C5a are generated during *in vitro* cultures of human T cells responding to allogeneic dendritic cells (DCs) (27). Both partners express the receptors for C3a and C5a (124–127), and C3aR- and C5aR-antagonists inhibit human T-cell proliferation, whereas recombinant C3a/C5a promotes alloreactive human $CD4^+$ T-cell expansion. Various subsets of human DCs produce complement, and C5aR/C3aR signaling regulates DC activation and function (27). Pharmacologic C5aR blockade reduced human anti-mouse graft-versus-host disease scores, inhibited T-cell responses in NOD/SCID/γc^{null} mouse recipients of human PBMCs, and enhanced stability of iTreg, verifying that the C5aR-dependent effects on human T cells apply *in vivo* (12,27). In further support of a role for local complement in human transplantation, the quantity of RNA message for alternative pathway complement components and receptors is higher in human allograft biopsies with histologic evidence of rejection compared with noninjured control tissue (122,128). Together, these translational findings provide proof-of-concept that C3a/C3aR and C5a/C5aR ligations are viable targets for facilitating prolonged survival of human transplants.

Conclusion

The complement system is a pathophysiologic mediator of kidney disease in humans. Building on the notion that plasma complement functions as an effector mechanism of antibody-initiated kidney injury, the recognition that kidney-derived and immune cell–derived complement participates in innate and adaptive immune responses and the appreciation that disorders of complement regulation underlie multiple kidney diseases have altered fundamental paradigms of complement biology. Ongoing translational immunology efforts along with the development of pharmacologic agents that block human complement components and receptors (129) now permit testing of the intriguing concept that targeting complement in patients with an assortment of kidney diseases has the potential to abrogate disease progression and improve patient health.

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Dendritic Cells and Macrophages: Sentinels in the Kidney

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Abstract

The mononuclear phagocytes (dendritic cells and macrophages) are closely related immune cells with central roles in anti-infectious defense and maintenance of organ integrity. The canonical function of dendritic cells is the activation of T cells, whereas macrophages remove apoptotic cells and microbes by phagocytosis. In the kidney, these cell types form an intricate system of mononuclear phagocytes that surveys against injury and infection and contributes to organ homeostasis and tissue repair but may also promote progression of CKD. This review summarizes the general functions and classification of dendritic cells and macrophages in the immune system and recapitulates why overlapping definitions and historically separate research have created controversy about their tasks. Their roles in acute kidney disease, CKD, and renal transplantation are described, and therapeutic strategy to modify these cells for therapeutic purposes is discussed.

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Introduction: General Roles of Dendritic Cells and Macrophages

All nonlymphoid tissues, including the kidney, harbor an intricate network of tissue-resident mononuclear phagocytes, which include dendritic cells (DCs) and macrophages. Macrophages were originally described >150 years ago by Slavjanski and Metchnikoff, and DCs were described by Steinman in 1973. These cell types are ontogenically related, and both serve sentinel roles but possess distinct hallmark functions. Macrophages are professional phagocytic cells that maintain tissue homeostasis (for example, by removing apoptotic cells) (Figure 1). During infection, they combat microbes by phagocytosis and production of toxic metabolites. Furthermore, they alert other immune cells by secreting proinflammatory chemokines and cytokines (1).

The canonical function of DCs is the activation of T cells (2). To this end, they gather antigens in tissues and transport them to draining lymph nodes to present them to specific T cells (3) (Figure 1). Immunogenic T-cell activation requires the DCs to have sensed pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs; for example, bacterial cell wall components, like LPS). Without sensing such patterns, DCs remain in an immature functional state and cause antigen-specific T cells to undergo apoptosis. This is one major form of so-called peripheral tolerance, which serves as a second checkpoint (after thymic tolerance) against T-cell responses against autoantigens that are usually devoid of PAMPs (4). By contrast, DCs that have matured after sensing PAMPs or DAMPS can induce vigorous proliferation of specific T-cell clones, which are released into the circulation to infiltrate infected tissues to combat the pathogens. In addition to DCs that migrate into lymph nodes, there are tissue-resident DCs that regulate infiltrating effector T cells by producing cytokines and chemokines (5,6).

As a rule of thumb, macrophages are innate immune effector cells, and DCs induce adaptive immunity. Their sentinel and regulatory functions overlap, and the functionally dominant cell type depends on their tissue of residence. In this review, we will summarize the phenotypical and functional characteristics of macrophages and DCs in the kidney and describe their role in acute renal injury and CKD.

Ontogeny and Subsets of DCs and Macrophages

Several DC classification system have been defined in mice and humans on the basis of the expression of subset markers and functional properties (Table 1). Many authorities distinguish CD11b-like myeloid DCs (mDCs), CD8-like mDCs, Langerhans cells (important only in the skin and not further considered here), plasmacytoid DCs (pDCs), and inflammatory DCs (7–9). mDCs and pDCs are derived from a distinct DC precursor in the bone marrow, whereas inflammatory DCs, like inflammatory macrophages, originate from monocytes (10). The CD11b-like mDCs preferentially activate $CD4^+$ T helper cells, the CD8-like mDCs are specialized at activating $CD8^+$ cytotoxic T cells, and pDCs induce direct antiviral immunity by secreting type I IFNs. Inflammatory DCs can perform all of these functions and serve as a rapid supply of additional DCs in situations of need, such as infections. For more information on these important functions of DCs in mice and humans, we refer to recent excellent reviews (6,9,11).

Macrophages have been classified in the last decades according to the M1/M2 or the classic/alternative

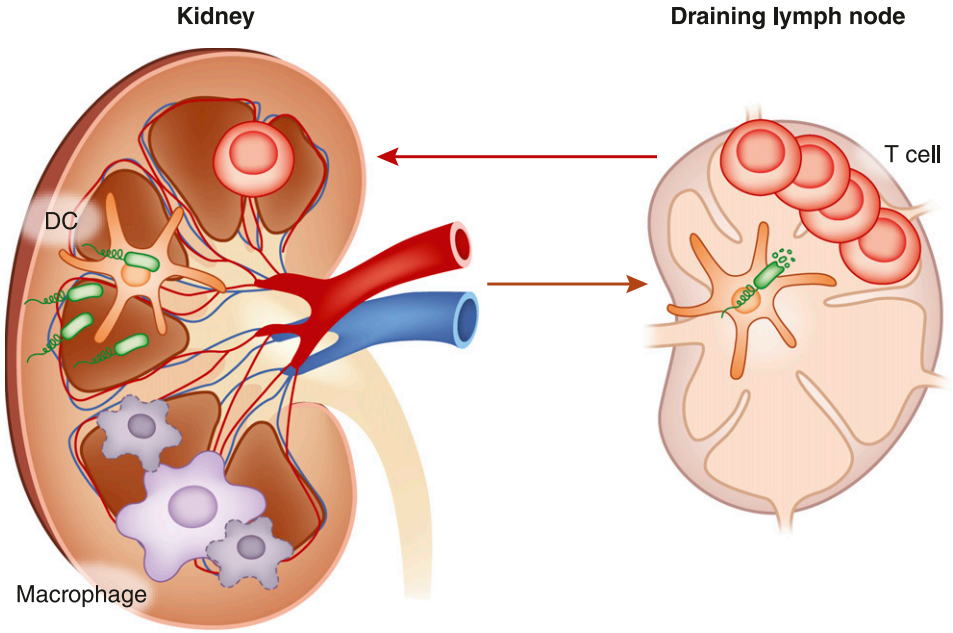

Figure 1. | **Canonical functions of renal dendritic cells (DCs) and macrophages.** Kidney DCs (orange) gather antigens (here represented in green as microbes) and transport them to draining lymph nodes to present them to specific T cells (red). Tissue-resident DCs can regulate activated T cells. Macrophages (violet) maintain tissue homeostasis, for example, by removing apoptotic cells. During infection, they may combat microbes by phagocytosis.

activation dichotomy, with the former performing proinflammatory antibacterial responses and the latter being involved in antiparasite immunity and tissue repair (12–14). However, these functions seem to represent merely two ends of a broad spectrum of macrophage polarization states (15). Another classification system uses the Ly6C marker to distinguish inflammatory and tissue-resident macrophages, which seem to perform distinct tasks in the anti-infectious defense (16,17). This difference is also evident in blood monocytes: in addition to the classic Ly6C$^+$ inflammatory monocytes that are dependent on the chemokine receptor CCR2, a distinct subset of Ly6C$^-$ monocytes expressing high levels of the chemokine receptor CX_3CR1 has been described (18). The latter seem to act as vascular sentinels that survey endothelial surfaces and may cause vasculitis and organ damage, which was explicitly shown in the kidney (19).

Recent work showed numerous other functional states of macrophages (20,21). This heterogeneity is further complicated by the discovery that certain tissue-resident macrophages, such as microglia and Langerhans cells, originate from not only the bone marrow but also, primitive precursors in the yolk sac and perhaps, the fetal liver (22,23). This finding has major implications for therapeutic strategies, because macrophages derived from primitive precursors seed organs before birth and multiply by local proliferation rather than recruitment from the circulation (24,25). Obviously, tissue-resident macrophages cannot be targeted by preventing their tissue recruitment in contrast to bone marrow–dependent macrophages. The classification of human tissue DCs and macrophages is less developed than that in mice because of technical limitations (21).

Discrimination between DCs and Macrophages

The realization that macrophages can also perform DC functions to a certain degree and *vice versa* has caused much uncertainty and debate about the exact demarcation between these two cell types. To understand this ambiguity, one has to remember that the fields of DC and macrophage research have historically developed more or less independent from each other. The marker molecules used to identify these cells considerably overlap; most notably, the murine DC marker CD11c used over three decades to distinguish DCs from macrophages is known to also be expressed by certain macrophages (for example, in the lung or the splenic marginal zone) (9,23). Likewise, the macrophage marker F4/80 is expressed by DCs in most nonlymphoid tissues, including the kidney (26) (Table 1). The systems commonly used to deplete DCs or macrophages for loss-of-function *in vivo* studies are often on the basis of these markers, and consequently, they are not specific either (27). Thus, many studies addressing either tissue macrophages or tissue DCs were unknowingly studying the same cells, often neglecting knowledge from the other discipline. In fact, the same mononuclear phagocyte may simultaneously fulfill the current criteria used by the DC community and those used by the macrophage community, because their definitions are not mutually exclusive.

Of course, semantic debates are of little importance to clinicians, let alone to patients. Therefore, many serious immunologists are currently discussing how to replace the numerous coexisting classification systems with a new system that is acceptable to both communities (28). Such a system will likely leave the current DC-macrophage dichotomy behind and encompass ontogenetic and functional properties. Before a consensus is reached, we will here adhere to

Table 1. General characteristics of dendritic cells and macrophages

Renal DC Subsets	Precursor Chemokine Receptors	General Functions	Key Markers Mouse	Key Markers Human	References
Myeloid/CD11b$^+$- like DCs	Common MDPs in the BM; CCR7, CCR2	Tissue surveillance; immune tolerance Ag transport to LNs, activation of T helper cells; recruitment of other immune cells to the kidney in GN and PN	CD11c^{high} F4/80$^+$ CX3CR1$^+$ CD11b$^+$	CD11c^{high} F4/80$^+$ BDCA-1$^+$	9,11
Crosspresenting DCs/ CD8α^+-like DCs	CDP XCR1$^+$	In renal LNs, CTL crosstolerance; very sparse in the kidney in lymph node T-cell activation; role within the kidney is unclear	CD11c^{high} CD11b$^-$ CLEC9A$^+$ XCR1$^+$ CD103$^+$	CD11c^{high} BDCA-3$^+$ CD14$^-$ CD1a$^+$	41,114
Plasma-cytoid DC	CDP	Possibly viral clearance through type I IFN; potential role in lupus nephritis	CD11c^{int} CD11b$^-$ CD8α^- B220$^+$ Gr1$^+$	BDCA-2$^+$ CD11c$^-$	115
Monocyte-derived/ inflammatory DC	Monocyte-derived	Innate defenses against Tip-DCs proinflammatory cytokine production	CD11c$^+$ CD11b$^+$ F4/80$^+$ Ly6C$^+$	DC-SIGN/CD209a$^+$ requires additional study	9,10
Inflammatory macrophages/ Ly6C$^+$ macrophages	CCR2$^+$ MDP	Displays high phagocytic activity; cytokine release	F4/80$^+$ Ly6C$^+$ CD11c$^-$	CD14^{high} CD16$^-$	10
Ly6C$^-$ macrophages	CX3CR1$^+$	Sentinel function; initiation of inflammatory response	F4/80$^+$	CD14$^+$	22,24
	MDP	Maintenance of tissue homeostasis; clearance of cell debris	Ly6C$^-$	CD16$^+$	
	Yolk sac derived		CD11c$^-$		

DC, dendritic cell; MDP, monocyte–DC precursor; BM, bone marrow; Ag, antigen; LN, lymph node; PN, pyelonephritis; CDP, common–DC precursor; CTL, cytotoxic lymphocytes.

the current nomenclature, which classifies renal mononuclear phagocytes expressing CD11c as DCs and those lacking this marker as macrophages.

Pattern Sensors on DCs and Macrophages

DCs and macrophages express a great variety of receptors to sense microbial PAMPs. The best studied examples are the Toll-like receptors (TLRs), which detect cell wall components of gram-positive and -negative bacteria, flagellin, and bacterial and viral RNA and DNA. In addition, TLR2 and TLR4 also recognize self-components that are normally sequestered but released in situations of stress, sterile inflammation, or cellular damage. Such DAMPs signals generally include molecules like HMGB1, heat shock proteins, or fibronectin. DAMPs with more or less specificity to the kidney include uromodulin (Tamm–Horsfall protein), hyaluronan, and biglycan (29–31).

Table 2 lists these classes and additional classes of pattern-sensing receptors expressed by DCs and macrophages such as lectins, like the mannose receptor, dectins, or DC-SIGN, which recognize carbohydrate moieties, especially of microbial origin (32). Retinoic acid-inducible gene 1–like helicases are intracellular sensors that detect the presence of viral nucleic acids in the cytoplasm, whereas nucleotide-binding oligomerization domain receptors-like receptors recognize intracellular bacterial PAMPs (33). Much attention has recently been given to the NLRP3 inflammasome (Figure 2A), a large molecular complex that, in response to various types of crystals (*e.g.*, silica, asbestos, cholesterol, Alzheimer fibrils, and islet amyloid polypeptide), proteolytically activates in phagocytes IL-1, an inflammatory master regulator (34). Numerous other receptors deliver additional information (for example, cytokine receptors that inform about the activation state of neighboring cells, metabolic sensors that detect consumption of nutrients, or even neuronal synapses that provide a direct link to neural regulation). DCs and macrophages integrate all this information, respond with activation or maturation, and become proinflammatory. Numerous experimental studies have examined the role of these sensors in models of various diseases, including those affecting the kidney, and promising candidates for therapeutic intervention have been identified (35). However, most of these studies used mice ubiquitously deficient for these sensors, and therefore, it is unclear whether an observed beneficial affect can be attributed to DCs or macrophages.

DCs and Macrophages in the Kidney

The tubulointerstitium of healthy kidneys contains numerous $CD11c^+$ cells with extensive dendritic protrusions that can be classified by phenotypic and functional properties as tissue DCs of the CD11b type (Figure 3, Table 1) (26,36). Most of these DCs comply with some definitions of tissue macrophages (for example, in terms of F4/80 expression) (21,37). A recent detailed study showed that the murine kidney contains at least five discrete subpopulations of mononuclear phagocytes, which cannot be simply classified into the conventional entities, because they performed both traditional DC and macrophage functions to differing degrees (38). Cells with predominant DC functionality are more abundant in the cortical tubulointerstitium but mostly absent from the glomeruli (39,40). Only 5% of the tubulointerstitial DCs belong to the CD8-like subset that has exclusive DC functionality (41), but their role in the kidney is unknown. Cells with unambiguous macrophage phenotype can be found in small numbers within glomeruli (42) and in the subcapsular and periarterial connective tissues (43,44). Renal macrophages expressing very high levels of F4/80 originate mostly from the yolk sac (22) and are more abundant in the medulla (40). Renal mononuclear phagocytes are partially derived from common DC precursors and monocytes (45).

Most of these observations were made in mice, where numerous experimental tools are available. Extrapolation to the human system is not straightforward, because the subset markers differ substantially between species. This may be one reason why, until now, only a few studies have examined DC and macrophage subsets in human kidney biopsies (46–48). These revealed new insight into old

Table 2. Selected pattern sensors relevant in kidney disease

Receptor	MPh Versus DC	Disease	Functional Role	Reference
TLR3/7/9	MPh	Lupus model (MRL-Fas mice)	Harmful	116
TLR9	MPh	Lupus model (MRL-Fas mice)	Harmful	117
TLR4	DCs and MPh	Urinary tract infection	Protective	53
NLRP3, inflammasome	DC	Oxalat-induced AKI	Harmful	35,87
SIGIRR	DCs	IRI	Protective	62
A2AR	MPh	IRI	Protective	118
AT1R	MPh	Renal injury in obesity	Harmful	84
IL-17R	DCs and MPh	UUO	Harmful	85
TREM1	MPh	UUO	Harmful	86
uPAR	MPh	UUO	Anti-inflammatory but profibrotic	119

TLR, Toll-like receptor; SIGIRR, single Ig IL-1–related receptor; uPar, urokinase receptor; NLRP3, NACHT, LRR, and PYD domains containing protein 3; A2AR, adenosine 2a receptor; AT1R, type I angiotensin receptor; TREM1, triggering receptor expressed on myeloid cells 1; MPh, macrophage; UUO, unilateral ureteral obstruction; IRI, ischemia reperfusion injury.

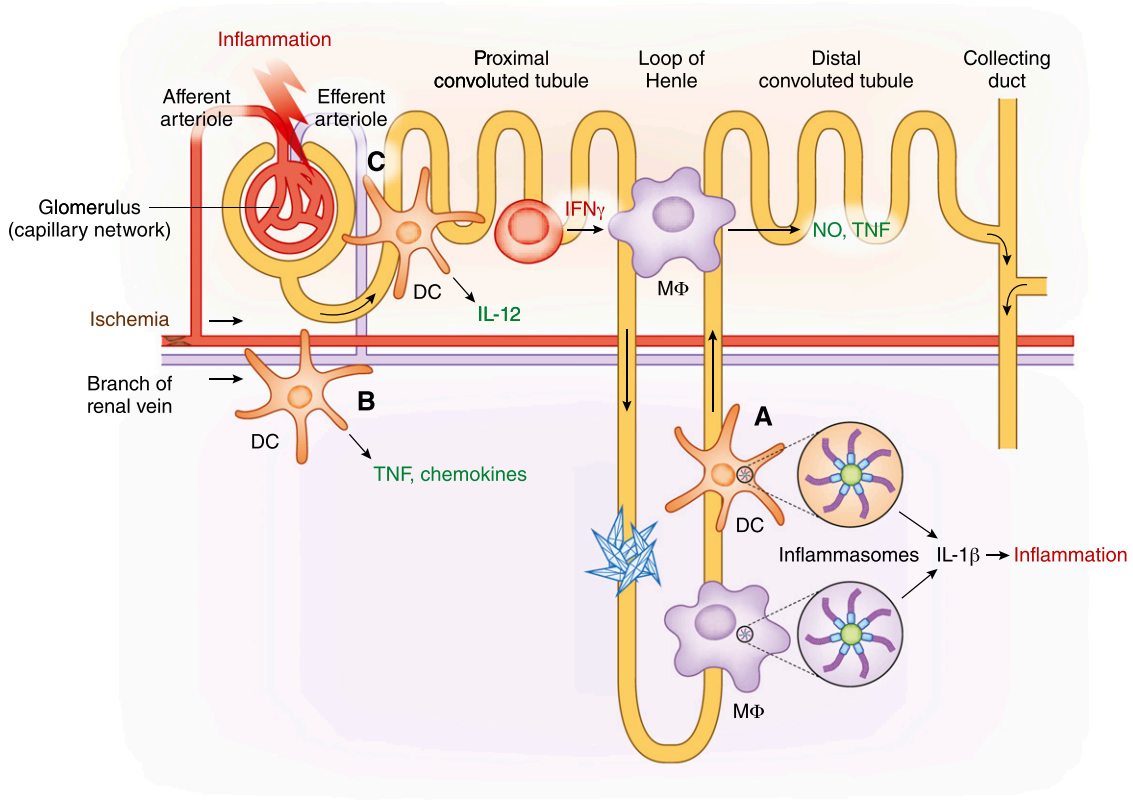

Figure 2. | **Role of renal DCs and macrophages in selected kidney diseases.** (A) Inflammasome activation in DCs and macrophages results from intratubular crystal formation, which is a consequence of the high osmolarity in the medulla that favors crystal formation. The inflammasome is an intracellular multimeric enzyme complex that contains adaptor proteins and caspase 1, which proteolytically activates IL-1β, resulting in inflammation. (B) Ischemia reperfusion injury activates DCs to produce TNF and chemokines that attract and stimulate injurious immune effector cells. (C) Glomerular inflammation activates periglomerular DCs in the renal cortex. They capture and present deposited or filtered antigens to CD4 T helper cells and stimulate these cells with IL-12. T cells secrete IFN-γ and cause macrophages to produce injurious mediators, like TNF or nitric oxide (NO). DCs, dendritic cells; MΦ, macrophage.

disease entities, such as shifts in the distribution and numbers of myeloid cells (identified in humans by the BDCA-1 marker) in lupus nephritis and necrotizing GN (47). There is great potential in this area to improve the histopathologic diagnosis of nephritis.

Homeostatic and Anti-Infectious Sentinel Functions

Maintaining Immune Tolerance against Renal Antigens

Kidney DCs constantly probe their environment using their dendrites, suggesting a sentinel role (26,36,46,49). Indeed, DCs in healthy kidneys continuously sample glomerular and tubular self-antigens and small molecular weight antigens that can constitutively pass the glomerular filter from the tubular lumen (50). It is unclear whether DCs do so by extending dendrites between epithelial cells into the tubular lumen as described for the intestine (51). In the absence of PAMPs, DCs express the suppressive molecule PDL-1 that induces apoptosis in T cells specific for such antigens (52). This function may serve to maintain immunologic tolerance against renal autoantigens or innocuous circulating small molecular weight antigens. However, this tolerance mechanism is undermined in diseases associated with proteinuria. When the glomerular filter becomes leaky, kidney DCs receive more proteins and in addition, high molecular weight antigens that stimulate potentially harmful T cells—another way that proteinuria can injure the kidney.

Defense against Urogenital Tract Infections

Renal DCs and macrophages use TLR4 to detect uropathogenic *Escherichia coli* (53,54), the most prevalent cause of kidney infections. DCs respond by producing chemokines that recruit neutrophilic granulocytes into the kidney to combat the bacteria by phagocytosis and production of toxic mediators (55) (Figure 4A). The DCs in the renal medulla are particularly potent at recruiting neutrophils (40), perhaps because they are the first to encounter the ascending bacteria and/or because of microenvironmental cues that cause them to specialize at anti-infectious functions. Notably, kidney macrophages contributed to neither the chemokine production nor the phagocytosis of uropathogenic *E. coli* (55). By contrast, another study reported that renal macrophages are critical for the defense against candida (56), which preferentially infect murine kidneys. The phenotype of the macrophages in that study (MHC II$^+$, F4/80$^+$, and CX$_3$CR1$^+$) resembles that of the DCs examined in the *E. coli* study, highlighting the current nomenclature

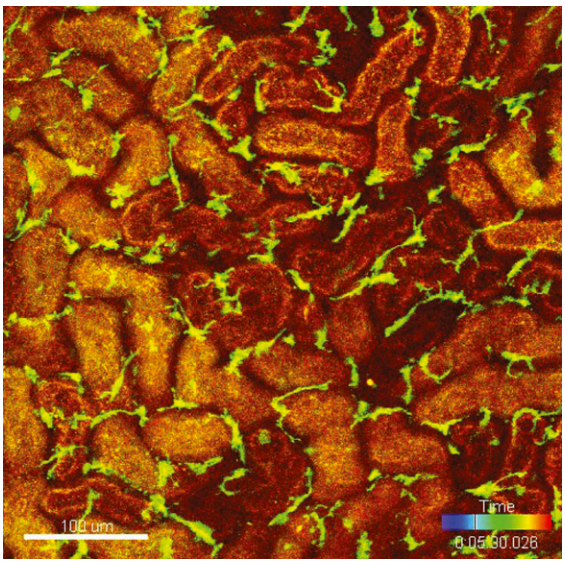

Figure 3. | **The renal mononuclear system.** Two-photon microscopy image of the renal mononuclear phagocyte system visualized by transgenic reporter mice in which cells, which include DCs, macrophages, and monocytes, harboring the receptor CX_3CR1 express green fluorescent protein. Reprinted from A. R. Kitching, with permission.

ambiguity resulting from overlapping definitions as described above.

By contrast, the defense against bacterial cystitis entirely depended on macrophages (more exactly on the interplay between two functionally distinct macrophage subsets that can be distinguished by expression of the Ly6C marker) (16) (Figure 4B). Bladder-resident $Ly6C^-$ sentinel macrophages performed a role comparable with the role of DCs in the kidney: they sensed the infection and produced chemokines that recruited neutrophils and $Ly6C^+$ monocytes. The latter did not directly combat bacteria but instead, performed an innate immune helper cell function. After sensing the infection, they produced the cytokine TNF as a helper signal that caused the sentinel macrophages to produce additional chemokines to guide neutrophils into the frontline of infection (the uroepithelium) to combat the bacteria. These findings showed that the immune system bases the decision of whether to induce antibacterial innate immunity on the agreement between several immune cells, probably to reduce the likelihood of false-positive decisions that would cause collateral damage—a principle well known from T- and B-cell responses of the adaptive immune system. These findings also help to explain why TNF is so important in antibacterial infection and why TNF-blocking therapies cause exacerbation of bacterial infections, including urogenital tract infections (57). It is unclear whether similar collaboration also occurs in the kidney during the defense against pyelonephritis. The finding that different myeloid cells act as sentinels in bladder and kidney highlights the close relationship between DCs and macrophages and the importance of microenvironmental factors that shape the local immune system. Exact knowledge of the mechanisms of antibacterial defense is important for designing superior therapies with minimal side effects.

Roles in Kidney Disease

AKI

Acute and chronic kidney inflammation is usually associated with the intrarenal accumulation of DCs and macrophages. Macrophages are widely considered important sources of proinflammatory cytokines and injurious mediators in various types of acute kidney diseases (58). In ischemia reperfusion injury (IRI), an important problem in kidney transplantation, kidney-resident DCs are the first to produce proinflammatory chemokines and cytokines like TNF (59), suggesting a proinflammatory role (Figure 2B). However, functional studies showed that kidney DCs prevented excess ischemic tissue damage (60,61), which has been linked to anti-inflammatory signaling mechanisms involving IFN regulatory factor 4 and immunosuppressive mediators, like IL-10 and single Ig IL-1–related receptor (62). Protective functions of DCs have also been shown in models of drug-induced tubulotoxicity (63) and acute crescentic GN (64), which may be mediated through the inducible costimulatory ligand on DCs (65), a T-cell suppressor. However, these studies have to be interpreted with caution, because they were obtained with the use of experimental techniques that may be associated with systemic neutrophilia as a side effect (66). Some studies may need to be revisited to clarify an influence of side effects.

After acute injury, both DCs and CSF-1–dependent macrophages orchestrated tissue repair by producing mediators like IL-22 (67,68), suggesting novel therapeutic avenues in both transplantation and acute tubular necrosis.

Chronic Progression of GN

Persistent signaling through pattern-sensing receptors causes resident DCs to mature and become proinflammatory. Furthermore, chronic kidney inflammation attracts circulatory monocytes through chemokines. These differentiate within the kidney into DCs and macrophages with proinflammatory functionality, which progressively replace resident immature DCs. Thus, the renal environment turns more and more proinflammatory and supportive of CKD progression. This functional change has been described in murine models of crescentic GN (40) and lupus nephritis (69). Matured DCs stimulated T helper cells, which produced cytokines like IFN-γ to activate renal macrophages to mediate kidney damage (Figure 2C), consistent with a type IV delayed type hypersensitivity reaction (70). This immune cell cross-talk may be the mechanistic explanation for the well known mononuclear periglomerular infiltrates regularly observed in many forms of GN (71). It is well established that the extent of tubulointerstitial mononuclear infiltration correlates with the kidney function and its prognosis in many types of CKD, including those not primarily by the immune system (like diabetic or hypertensive nephropathy) (71), suggesting a general role of tubulointerstitial immune cell cross-talk in CKD progression. Thus, the histopathologic diagnosis of nephritis may be advanced by considering functional parameters, like the DC maturation state (72), the T cell cytokine production, or macrophage activation.

Role in Lupus Nephritis

Immune cell cross-talk is also important in lupus nephritis, a renal manifestation of a systemic immune disorder resulting from a defect in the homeostatic clearance of apoptotic

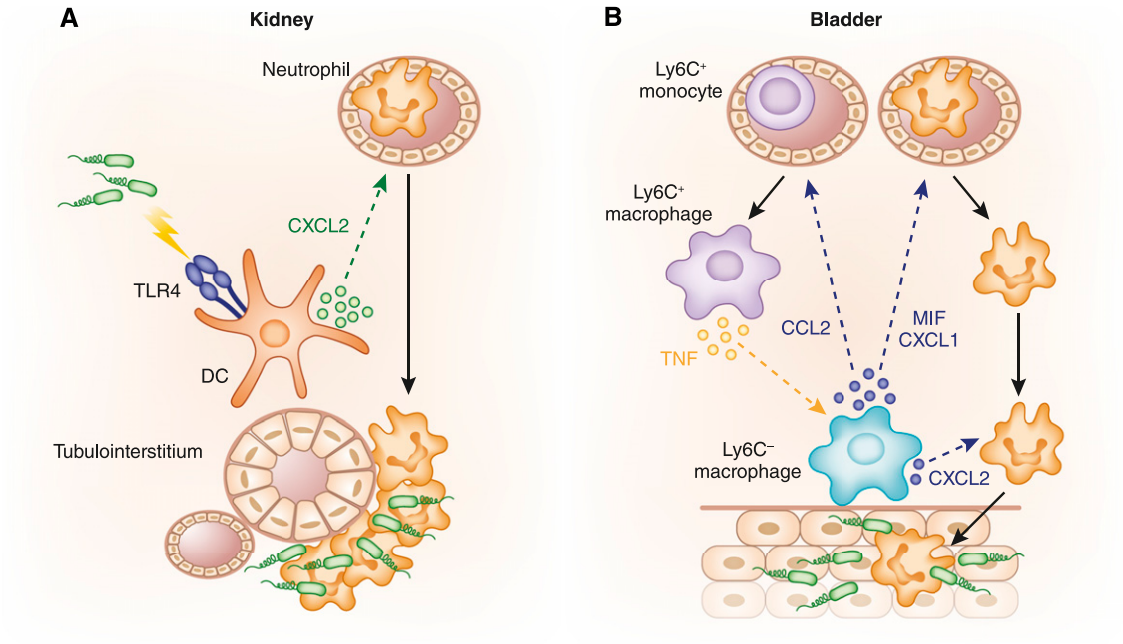

Figure 4. | **Immune response against urinary tract infection.** (A) In bacterial pyelonephritis, ascending uropathogenic *E. coli* (green) is sensed by Toll-like receptor (TLR) -expressing kidney DCs positioned in the medulla adjacent to collecting ducts and tubules. Medullary DCs respond by secreting CXCL2 that attracts neutrophils to eliminate the bacteria. (B) In bacterial cystitis, resident Ly6C$^-$ sentinel macrophages sense uropathogenic *E. coli* and start secreting chemokines to recruit circulatory neutrophils and Ly6C$^+$ monocytes. The latter differentiates into Ly6C$^+$ helper macrophages that release TNF as helper signal, which enables Ly6C$^-$ sentinel macrophages to produce additional chemokines to guide neutrophils into the frontline of infection (the uroepithelium) to combat the bacteria. MIF, macrophage migration inhibitory factor.

cells (73), sensing of the accumulating self-nucleic acids that mimic a viral infection (74), and the ensuing loss of immune tolerance to nuclear autoantigens (75,76). In addition to mDCs, pDCs also play an important role in this condition by sensing nucleic acids antigens using TLR7 and TLR9 and driving intrarenal inflammation by secretion of type 1 IFN (74). Lupus nephritis is one of two conditions in which DCs infiltrates in glomeruli have been described (77), the other one being ANCA-associated GN (78).

Role in Nonimmune CKD

DCs play a minor role in CKD driven by mechanic or toxic injurious mediators. The best studied example is unilateral ureteral obstruction, the standard kidney fibrosis model. Although both DCs and macrophages showed signs of activation, only the removal of macrophages (but not DCs) attenuated kidney fibrosis (79,80). The important functional role of macrophages explains the correlation between their intrarenal numbers and the severity of fibrosis in human studies (81). Mechanistically, activated macrophages secreted reactive oxygen species and proinflammatory cytokines, which induced apoptosis of tubular epithelial cells and stimulated interstitial fibroblasts to deposit extracellular matrix, leading to kidney fibrosis (82,83). Several macrophage-activating receptors have been described with inhibition that may be of therapeutic value in nonimmune CKD (84–86) (Table 2).

Intrarenal Inflammasome Activation

Many forms of sterile inflammation are driven by inflammasome activation and important for the nephrologist, usually lead to tissue fibrosis (34). The kidney is predisposed to inflammasome activation, because the concentration of the primary filtrate and the high osmolarity in the renal medulla favor crystal formation after the solubility coefficient of a given salt is exceeded within the tubular lumen (Figure 2A). Indeed, it has been reported that calcium oxalate crystals activate the inflammasome in renal DCs (87), which may cause progressive loss of kidney function over weeks (88). Additional examples of inflammasome-activating nephrotoxic crystals are formed by uric acid (resulting in gout nephropathy) and adenine (89), which is released, for example, during chemotherapy. Experimental studies have also shown a role of the inflammasome in IRI (90) and unilateral ureter ligation (91). Numerous stimuli other than crystals have been reported to trigger the inflammasome, but it is unclear which of them are important in these conditions. Regardless of the underlying reasons, inhibition of the inflammasome product, IL-1, has recently been used successfully in many forms of sterile inflammation (92), albeit not yet in those affecting the kidney. Furthermore, small molecular weight inhibitors of the inflammasome itself are currently being developed and tested. Finally, it might be possible to prevent inflammasome activation by targeting the renal cells in which it is expressed (the DCs and macrophages).

Kidney Transplantation

Renal allograft rejection results from the recognition of graft antigens that provoke an immune response of the recipient (93). DCs are central to both the direct and indirect allorecognition pathways, where donor antigens are presented

to recipient T cells by donor or recipient DCs, respectively. Direct recognition may contribute to acute allograft rejection (94), whereas indirect recognition causes chronic allograft rejection and alloantibody formation (95). This is logical, because recipient B cells need to receive recipient T-cell help, which can be stimulated only by recipient DCs. Donor DCs possess a limited half-life and can contribute to rejection only in the acute phase. During that phase, donor DCs are exposed to IRI-mediated damage that renders them particularly stimulatory. IRI can be studied in animals much more easily than full organ transplantation, and therefore, abundant information is available on the molecular mechanism of IRI, showing, for example, the potent production of proinflammatory cytokines (59). Consistently, the analysis of human kidney biopsies showed that a strong mDC influx during acute rejection predicts a poor outcome (96).

Therapeutic Manipulation of Renal DCs and Macrophages

Adoptive Transfer of DCs and Macrophages

The transfer of tolerogenic donor-derived immature DCs before kidney transplantation seems to be a promising therapeutic strategy to prevent proinflammatory functions of kidney passenger DCs. Such donor DCs can promote alloantigen-specific T-cell unresponsiveness and transplant survival (97). Inhibition of T effector cells and induction of regulatory T cells were observed in volunteers treated with DCs (98,99). These effects may be augmented by immunosuppressive therapies (100).

Transfer of activated macrophages as Ehrlich's magic bullets that migrate into sites of inflammation has been shown to aggravate kidney disease (101), consistent with the proinflammatory function of these cells. By contrast, the transfer of macrophages genetically modified to express immunosuppressive mediators attenuated inflammatory kidney disease (102,103). It remains to be seen whether such genetic modifications are stable and safe enough for therapeutic use in humans.

Removal of DCs and Macrophages

The depletion of renal DCs and macrophages is the most straightforward approach to prevent their disease-aggravating effects in CKD. In animals, this can be achieved with the use of clodronate liposomes, which are not specific for DCs or macrophages. More specific are the numerous transgenic tools previously used *in vivo* for loss-of-function experiments, which allow determination of how disease models develop in the absence of DCs and/or macrophages. However, current techniques do not permit their selective removal from the kidney to preserve their anti-infectious activity in other organs. Additionally, it is unclear whether the ensuing side effects would be tolerable in humans.

Preventing DCs and Macrophage Recruitment

DC and macrophage recruitment into the inflamed kidney can be inhibited by blocking the chemokine receptors involved. The receptor CX_3CR1 mediates the homeostatic colonization of the kidney (but not other organs) with DCs, except the intestine (40). Therefore, CX_3CR1 should represent a highly desirable therapeutic target, because its inhibition should entail fewer side effects in other organs. However, under inflammatory conditions, CX_3CR1 is not the only receptor mediating monocyte recruitment; also, CCR1, CCR2, and CCR5 have been implicated (104,105). It remains to be seen whether and how much these receptors can compensate when CX_3CR1 is blocked. Particularly, CCR2 is important for proinflammatory macrophages, and its inhibition (or inhibition of its ligand CCL2) was effective in mouse models of unilateral ureter ligation (79), lupus nephritis (106), ischemia/reperfusion (107), and diabetic nephropathy (108). Chemokine receptors can be targeted well by small molecular weight drugs; some of these are currently under investigation, but their value for treating kidney disease is unclear at present. Finally, some immunosuppressive drugs, such as laquinimod, have been shown to reduce chemokine secretion and immune cell infiltration in other tissues and may be of use in nephritis (109).

Preventing DC Maturation and Macrophage Activation

Another promising approach is to inhibit DC maturation, the prerequisite for their ability to aggravate chronic disease. An obvious candidate is the transcription factor NF-κB, a master regulator of immune cell activation, which offers the additional advantage of also being active in two additional main components of renal mononuclear infiltrates (T helper cells and macrophages). NF-κB inhibitors have been used successfully in several immune-mediated diseases, including SLE (110), but have not yet been used successfully in primary CKDs. Interestingly, the effectivity of type I angiotensin receptor blockade in CKD is partially mediated through inhibition of macrophages, which express this receptor as well (111). Table 2 lists additional receptors important in CKD with the potential to modify DC or macrophage activation. Some of the immunosuppressive agents used to prevent transplant rejection prevented DC activation *in vitro* (for example, sirolimus [112] and tacrolimus [113]). However, their usefulness in CKD is limited by side effects. Finally, the numerous recent inhibitors of proinflammatory cytokines produced by DCs and macrophages possess therapeutic potential, but these drugs will be discussed in another review of this series.

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T Cells: Soldiers and Spies—The Surveillance and Control of Effector T Cells by Regulatory T Cells

Bruce M. Hall

Abstract

Traditionally, T cells were CD4$^+$ helper or CD8$^+$ cytotoxic T cells, and with antibodies, they were the soldiers of immunity. Now, many functionally distinct subsets of activated CD4$^+$ and CD8$^+$ T cells have been described, each with distinct cytokine and transcription factor expression. For CD4$^+$ T cells, these include Th1 cells expressing the transcription factor T-bet and cytokines IL-2, IFN-γ, and TNF-β; Th2 cells expressing GATA-3 and the cytokines IL-4, IL-5, and IL-13; and Th17 cells expressing RORγt and cytokines IL-17A, IL-17F, IL-21, and IL-22. The cytokines produced determine the immune inflammation that they mediate. T cells of the effector lineage can be naïve T cells, recently activated T cells, or memory T cells that can be distinguished by cell surface markers. T regulatory cells or spies were characterized as CD8$^+$ T cells expressing I-J in the 1970s. In the 1980s, suppressor cells fell into disrepute when the gene for I-J was not present in the mouse MHC I region. At that time, a CD4$^+$ T cell expressing CD25, the IL-2 receptor-α, was identified to transfer transplant tolerance. This was the same phenotype of activated CD4$^+$CD25$^+$ T cells that mediated rejection. Thus, the cells that could induce tolerance and undermine rejection had similar badges and uniforms as the cells effecting rejection. Later, FOXP3, a transcription factor that confers suppressor function, was described and distinguishes T regulatory cells from effector T cells. Many subtypes of T regulatory cells can be characterized by different expressions of cytokines and receptors for cytokines or chemokines. In intense immune inflammation, T regulatory cells express cytokines characteristic of effector cells; for example, Th1-like T regulatory cells express T-bet, and IFN-γ–like Th1 cells and effector T cells can change sides by converting to T regulatory cells. Effector T cells and T regulatory cells use similar molecules to be activated and mediate their function, and thus, it can be very difficult to distinguish soldiers from spies.

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Introduction

In this review, effector T cells are referred to as soldiers, because they mediate immunity and destroy cells with the specific antigen. Until recently, the role of T regulatory cells (Tregs) in monitoring and limiting every step of the effector immune response has been underappreciated. Although they are referred to as spies, their function is to not only monitor immunity but also, actively control immunity. Tregs prevent uncontrolled immunity, unnecessarily inflicting injury that, in its own right, may kill the host.

Whereas an antibody identifies extracellular structures, such as soluble antigen or antigen on surfaces of cells or organisms, T cells monitor the intracellular compartment of the host. They do this so that they can kill cells infected with a pathogen or cells that are allogeneic, xenogeneic, or malignant cells expressing new tumor-associated antigens. In autoimmunity, they kill normal cells. To deal with the vast array of pathogens, T cell responses, such as Th1, Th2, and Th17, have evolved to allow protective responses that are adapted to better eliminate the different types of pathogens.

Tregs are generated in all immune responses and limit response to pathogens, transplant tissue, and tumor cells. Normally, Tregs control autoimmune responses, and autoimmunity occurs when Treg responses fail. Tregs are beneficial in patients with transplants, because they can promote tolerance, whereas they are undesirable in cancer, where they prevent elimination of malignant cells by T cells. Thus, promoting Tregs in patients with transplants or autoimmunity is desirable, whereas in chronic infection and malignancy, it may be undesirable.

What Is a T Cell?

T cells are mainly produced in the thymus and were first recognized as lymphocytes that do not express surface Ig or genes for Ig (1). The hallmark of a T cell is expression of an antigen-recognizing T cell receptor (TCR) (2). There are two forms of TCRs: an α- and β-chain TCR (TCRα,β) expressed by 95% of peripheral T cells (3) and a γ- and δ-chain TCR (TCRγ,δ) (4). TCRγ,δ T cells will not be discussed further. Each T cell has a unique TCR with the potential to recognize a unique antigen. Progeny of T cells express the same TCR and are clonally expanded to effect antigen-specific immunity.

The TCR is coexpressed with CD3, a complex of heterodimers of CD3ε,γ and CD3ε,δ with a homodimer of ζ-chain. The negatively charged transmembrane regions of the CD3 associate with positively charged transmembrane regions of TCR. TCR has a small intracellular domain; thus, signaling after contact with a specific antigen is by CD3. CD3 is phosphorylated on

an immunoreceptor tyrosine-based activation motif that allows ζ-associated protein 70 to activate the intracellular pathway that releases calcium from the endoplasmic reticulum. Calcium binds to calmodulin to activate phosphatase activity of calcineurin to activated nuclear factor of activated T cells (NFAT). NFAT, a transcription factor, activates a series of genes, especially IL-2. Calcineurin inhibitors, such as cyclosporin and tacrolimus, block activation of T cells by inhibiting calcineurin activation.

Other molecules unique to T cells are CD2 and some isoforms of CD45. All other cell surface markers are differentially expressed on T cell subpopulations or non–T cells.

Presentation of Antigen to TCR

TCR, unlike antibody, does not directly bind to unprocessed antigen. TCR recognizes peptides of antigen presented by MHC present on cell membrane. The role of MHC molecules as presenters of antigen was first recognized when the crystal structure of human HLA-2 identified a peptide not encoded by the HLA gene in a groove created by the variant α1- and α2-domains (3,5,6).

Antigenic peptides presented by class I MHC molecules, such as HLA-A,B,C, are usually from proteins synthesized within a cell and bind to class I MHC before its expression on the cell surface (7).

The antigenic peptide in a class I MHC groove is usually nine amino acids. Each class I MHC only presents peptides with a consensus motif, usually at p2, p3, and p5 amino acids, that fits its groove. The antigenicity is generated by the amino acids at the other positions. Humans have six class I MHCs, two HLA-A, two HLA-B, and two HLA-C, with different consensus motifs that each can present thousands of different peptides. Thus, a cell can display many thousands of intracellular peptides in class I MHC, like a chip array (8). CD8 binds to the invariant α3-domain of class I MHC (9), facilitating TCR on $CD8^+$ T cells surveying antigen presented by class I MHC.

Antigenic peptides presented by class II MHCs (in humans, HLA-DR, HLA-DP, and HLA-DQ) are usually from proteins produced outside the cell. These foreign proteins are ingested and processed by class II MHC-expressing cells, such as dendritic cells, monocytes, macrophages, oligodendrocytes, Langerhans cells, and B cells. TCR recognizes antigenic peptides of ≥15 amino acids entrapped in a groove created by the variant α1- and β1-domains (10). CD4 binds to the invariant β2 of class II MHC to facilitate TCR recognition of antigenic peptides presented by class II MHC (11,12).

The TCR antigen recognition site interacts with both the peptide and the surrounding MHC structure. This explains MHC restriction of cytotoxic T cells, which only kills virally infected cells expressing the same class I MHC that activated the T cell (13).

The pathways for presentation of antigen by MHC are complex (7). To activate T cells, antigen-presenting cells (APCs) must first be activated by the antigen and induced to express MHC and costimulatory molecules. APCs are activated by bacterial wall molecules or virus materials, such as double-stranded DNA, that bind to Toll-like receptors (7). This leads to production of inflammatory mediators, such as TNF-α, IL-1β, and PGE2, which further activate APCs (7).

In nonimmune situations, MHC class II is only expressed by APCs and B cells. During immune inflammation, IFN-γ induces expression of class II MHC on somatic cells and increases class I MHC expression (14). Activated Tregs are the only T cells that express class II MHC (15), but its function on Treg is unknown.

Generation of Diversity in TCR for T Cells in Thymus—Clonal Deletion and Selection for Ability to React to Self-MHC

A massive number of different TCRs are generated when $CD4^+CD8^+$ thymocytes are produced. This occurs by random selection of different combinations of variable and junctional genes for α- and β-chains and diversity genes for β-chain. These form three hypervariable or complementarity determining regions that are the sites where TCRs interact with antigenic peptide and the MHC (6). The antigen recognition site of TCR interacts with the peptide and the surrounding self-MHC structure.

There is negative selection by clonal deletion of thymocytes with TCR that strongly recognize self-antigen (16–18), which leads to tolerance to self. Autoimmune regulator, a transcription factor, induces expression of a large number of proteins found in peripheral tissues in thymic medullary epithelial cells (19). Peptides from these normal proteins expressed in peripheral tissues are presented on self-MHC to thymocytes and promote deletion of autoreactive clones. Mutations in autoimmune regulator cause Autoimmune Polyendocrinopathy Syndrome type 1 with hypoparathyroidism, primary adrenocorticoid failure, and chronic mucocutaneous candidiasis (20). The mechanisms for deletion of autoreactive clones are not perfect, and surviving autoreactive T cells are normally controlled by peripheral mechanisms that prevent their activation, including by Treg.

In the thymus, there is also positive selection of T cells with TCR that can bind to antigen associated with self-MHC (21). If a TCR does not bind to self-MHC, the thymocyte dies. If a thymocytes TCR recognizes class II MHC, CD4 expression is retained, and CD8 expression lost. If the TCR recognizes class I MHC, the thymocyte continues to express CD8 but not CD4. Nearly all T cells released from the thymus express either CD4 or CD8.

The majority of peripheral TCRα,β T cells is effector programmed to become soldiers. A minority of peripheral $CD4^+$ TCRα,β T cells released from the thymus expresses CD25 and FOXP3, and they are professional Tregs or spies. Both effector T cells and Tregs have a vast array of TCR to recognize a broad repertoire of specific antigen.

Nonantigen-Specific Adhesion Molecules Required for *Signal 1* to Activate T Cells

LFA1, LFA2(CD2), and LFA3(CD58) were identified to facilitate cytotoxic T cells interaction with target cells (22) (Figure 1). CD2 binds to LFA3 expressed on APCs and other cells (23) and is widely expressed in the kidney (24). LFA1, an integrin heterodimer of CD11a and CD18, binds to intercellular adhesion molecule 1 (ICAM1) and is the initial contact of T cells with APCs. LFA1 is also expressed by B cells, macrophages, and neutrophils. ICAM1, although constitutively expressed by APCs, can be induced on other cells by IFN-γ (25). Antibodies to LFA1, LFA2, and LFA3 can

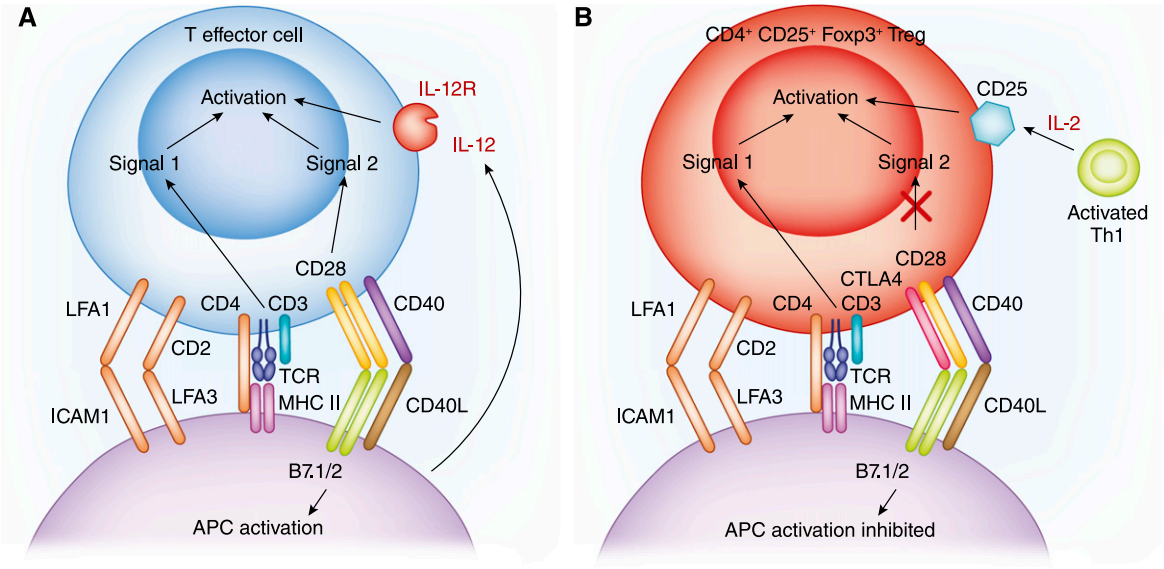

Figure 1. | **Activation of effector and regulatory T cells by antigen presenting cells.** Key surface molecules in activation of (A) T effector cells and (B) T regulatory cells (Tregs). The key molecules required for both cells are similar. The T cell receptor complex includes CD3, CD2, CD4 or CD8, LFA1, and CD45R, and activation of T cell receptor (TCR) by antigen results in *Signal 1* for T effector cells and Tregs. In effector T cell–lineage T cells, CD28 on the T cells is activated by B7.1 and B7.2 on antigen-presenting cells (APCs) and generates *Signal 2*, which combined with *Signal 1*, initiates effector T-cell activation. The activation of effector T cells is augmented by CD40L binding to CD40 and cytokines, such as IL-2 and IL-12, for generation of Th1 cells. With Tregs, CTLA4 binds to B7.1 and B7.2 and limits activation through CD28. Thus, the effector T cells *Signal 2* pathway is not required for Treg activation. The second signal for Treg activation is generated by IL-2 binding to the IL-2 receptor, which includes CD25.

delay or prevent rejection and are potential therapeutic targets in transplantation and autoimmunity.

These molecules form an immunologic synapse around the TCR/MHC interaction (26). The synapse includes TCR, CD3, CD4 or CD8, CD2, LFA1, and CD45 that collectively produce *Signal 1* for T-cell activation (Figure 1). *Signal 1* is blocked by calcineurin inhibitors, such as cyclosporin, which complexes with cyclophilin, or tacrolimus (FK506), which complexes with FK506 binding protein (FKBP). Both complexes inhibit calcium binding to calcineurin and the induction of phosphatase activity required to release NFAT.

The molecules and mechanisms of antigen recognition and generation of *Signal 1* required to activate antigen-specific T cells are common to effector T cells and Tregs (Figure 1).

Signal 2 for T Cell Activation

CD28 expressed by naïve T cells binds to B7.1(CD80) or B7.2(CD86) on APCs and generates *Signal 2* (27). B7.1 and B7.2 are normally only expressed by specialized APCs, such as dendritic cells and Langerhan's cells. These APCs need to be activated by a pathogen binding to Toll-like receptors to induce the inflammasome and production of IL-1β, IL-6, and TNF-α. This increases expressions of MHC and ligands on APCs that are required for T cells to bind. Normal healthy somatic cells cannot activate T cells, because they do not express B7.1 and B7.2. CTLA4 from Tregs preferentially binds and blocks to B7.1 and B7.2, preventing induction of *Signal 2*. mAbs to block costimulation have been used to prevent rejection. CTLA4-Ig (abatacept, and belatacept) blocks T-cell activation and prevents renal transplant rejection and some autoimmunity. Antagonists of CTLA4 (ipilimumab) block Treg function and allow immune destruction of tumors, such as melanoma.

Signal 2 activates a separate intracellular pathway in T cells that is blocked by target of rapamycin (mTOR) inhibitors, such as rapamycin, that also bind to FKBP. This complex of rapamycin/FKBP blocks activation of mTOR but not calcineurin. mTOR inhibitors act by blocking signal 2 and prevent rejection.

The combination of *Signal 1* and *Signal 2* induces expression of genes required for T cell activation and promotes T cell proliferation to produce effector T cells (Figure 1A). *In vivo* natural T regulatory cells (nTregs) cannot active *Signal 2* (Figure 1B), albeit *in vitro*, this pathway is activated by anti-CD28 to polyclonally expand nTreg.

CD40L is expressed by T cells and binds to CD40 on APCs, B cells, and macrophages as well as other cells. CD40L binding to CD40 activates the APCs that, in turn, activate T cells. Other T cell surface molecules promote APC activation, including inducible T cell costimulatory (CD278), a member of the CD28, CTLA4 family (28).

Naïve, Activated, and Memory T Cells

The T cells that have not previously contacted their relevant antigen are naïve. The normal immune system has a massive reservoir of naïve T cells, with the potential to respond to millions of different antigens presented by self-MHC. For a specific virus, $<0.01\%$ of naïve T cells have a specific TCR, whereas 1%–9% of naïve T cells have TCRs that recognize MHC on incompatible allografts.

Naïve T cells are programmed to recirculate from blood into peripheral lymphoid tissues and then back to blood

by the lymphatics to facilitate contact with their specific antigen (29). They traffic past APCs activated by antigen in tissues that migrated through the afferent lymphatics to lymphoid tissues. Naïve T cells that recognize antigen are arrested and activated by these activated APCs (30).

CD62L expressed on naïve T cells binds to ligands on high endothelial venules to facilitate this migration into lymphoid tissues (31). The chemokine receptor CCR7 on naïve T cells is bound by CCL21 and CCL19 from the lymphoid tissues to attract them (32). CD62L and CCR7 distinguish naïve from effector and memory T cells, which express other integrins, such as VLA4, and chemokine receptors that promote migration into inflamed tissue (Table 1). Activated T cells and effector memory T cells migrate through normal tissues (33) to survey for cells expressing specific antigen. Central memory cells express CD62L and CCR7 and migrate through lymphoid tissues, like naïve T cells. Other markers of memory T cells are expression of CD45RO, CD44, and higher expression of CD2 than naïve T cells.

Effector T cells and Tregs express the same markers and traffic in the same way (Table 1).

CD45, a Marker of T-Cell Activation.

CD45 is expressed by all leukocytes but not expressed by other cells. CD45 is encoded in 34 exons that are fully transcribed and glycosylated in a gp220 expressed by B cells and other leukocytes but not T cells. The intracytoplasmic domain of CD45 contains two tyrosine phosphatases that associate with kinase associated with TCR/CD3 and Ig signaling. CD45 is essential for antigen-driven activation of B and T cells and promotes their differentiation and proliferation. The ligand for CD45 is unknown.

On T cells, exons 4–6, which encoded CD45RA, CD45RB, and CD45RC, respectively, are spliced out to generate potentially eight different protein products. CD45RA(gp200kd) is expressed by naïve T cells. On activation of T cells, CD45RA is replaced by CD45RB, CD45RC, and/or CD45RO. In alloimmune responses, naïve T cells express CD45RB (34), and blocking CD45RB prevents rejection. Memory T cells only express CD45RO(gp180) where exons 4–6 are spliced out. The major component of antithymocyte globulin is anti-CD45.

Soldier Versus Spy T Cells

The majority of T cells expressing TCRα,β are programmed to be effector cells and express either CD4 or CD8 but do not express the IL-2Rα(CD25) or FOXP3 (35). A minority (<5%) population of professional spies is $CD4^+CD8^-CD25^+$ $FOXP3^+$ Tregs (35,36). Most early work on T cells focused on immune destruction of infected, malignant, transplanted, or normal self-cells in autoimmunity but not Tregs.

In the early 1970s, T cells that suppress immunity were described as $CD8^+I\text{-}J^+$ T cells. Thymocytes were suppressive, and removal of the thymus made animals prone to autoimmunity. When no gene for I-J was found in the murine MHC region, suppressor T cells fell into disrepute, and most work in the field was abandoned (37).

The revival of Tregs started when $CD4^+CD8^-$ T cells and not $CD8^+$ T cells were found to transfer antigen-specific tolerance and suppress naïve T effector cells (38). The $CD4^+$ T cells that transferred tolerance expressed CD25, the IL-2 receptor-α (15). This created a paradox, because $CD4^+$ T cells activated to mediate rejection expressed CD25 (39), and their depletion with mAbs to CD25 reduced rejection in animals (40,41) and humans (42). We now know that depletion of $CD25^+$ T cells prevents induction of tolerance in transplant and autoimmunity. Thus, the soldiers and spies had the same markers.

Other observations supported the existence of $CD4^+$ Tregs. First, transferred tolerant $CD4^+$ T cells interacted

Table 1. Comparison of phenotype of Th effector lines and Th-like T regulatory cells

Marker	Th1 Effector	Th1-Like Treg	Th2 Effector	Th2-Like Treg	Th17 Effector	Th17-Like Treg
CD4	+++	+++	+++	+++	+++	++
TCR/CD3	+++	+++	+++	+++	+++	+++
CD2	+++	+++	+++	+++	+++	+++
CD45	RO	RO	RO	RO	RO	RO
CD25	++	+++	−	+++	−	+++
FOXP3	−	+++	−	+++	−	+++
T-bet	+++	+++	−	−	−	−
IRF4	−	−	++	+++	−	−
RORγt	−	−	−	−	+++	++
Stat	1	1	4, 5	4	3	3
Chemokine receptor	CXCR3	CXCR3	CCR8	CCR8	CCR6	CCR6
IFN-γ	++++	+++	−	−	−	−
IL-5	−	−	++++	+++	−	−
IL-17A	−	−	−	−	+++	+
IL-2	+++	−	−	−	−	−
IFNGR	++	+++	−	−	−	−
IL-12Rβ2	+++	+++	−	−	−	−
IL-5Rα	−	−	−	+++	−	−

Treg, T regulatory cell; TCR, T cell receptor.

with a second host's $CD4^+$ T cells to induce transplant tolerance (43). Second, autoimmunity in neonatal thymectomized mice was prevented by $CD4^+CD25^+$ T cells (44). Third, in the early 2000s, the transcription factor FOXP3 identified Tregs from activated $CD4^+CD25^+$ T effectors (35,36). FOXP3 prevents IL-2 production and induces CD25 expression.

Defects in the FOXP3 gene lead to immunodysregulation polyendocrinopathy enteropathy X–linked syndrome manifesting as enteropathy, dermatitis, nail dystrophy, autoimmune endocrinopathy, lymphoid enlargement, and infections (45). Scurfy mice have defects in FOXP3, widespread uncontrolled lymphoid hyperplasia of $CD4^+$ T cells, T cell infiltration of organs, and overexpression of cytokines (46). Similar phenotypes to scurfy are found in CTLA4, IL-2, and CD25 knockout mice, indicating the key role that these molecules play in nTreg function.

The Survival and Maturation of T Cell Subpopulations Depends on the Cytokine Milieu

Different functional T cell subpopulations express different cytokine receptors and cytokines. Cytokine binding to its specific receptor induces Jaks, Stats, and cell line-specific transcription factors.

Expression of cytokine receptors distinguishes different subpopulations. Effector lineage T cells need IL-7 to survive and express IL-7Rα(CD127). $CD4^+CD25^+FOXP3^+$ Tregs express IL-2R and need IL-2 to survive. Tregs have low expression of CD127, and depletion of $CD127^{hi}$ cells is used to enrich Tregs and eliminate activated effector $CD4^+CD25^+$ T cells (47). Memory T cells are maintained by IL-15 and express IL-15Rα.

Activated T effector cells and Tregs express different cytokine receptors and cytokines. These patterns of expression distinguish different subpopulations (Figures 2 and 3).

Activation of Professional Soldiers—T Effector Cells

Effector lineage $CD4^+CD25^-CD127^{hi}FOXP3^-$ T cells activated by antigen are clonally expanded and can develop into functionally different $CD4^+$ T cell lines. Different pathways are driven by the cytokine milieu and the cytokine receptors induced during activation (Figure 2) and induce functional distinct T cells, such as Th17, Th1, Th2, or Tfh cells.

Th17 Cells

Th17 cells are induced if the inflammatory cytokine IL-6 (and IL-1β in humans) is present with TGF-β (48,49). The transcription factors Stat3 and RORγt are induced and regulate Th17 cytokine expression. TGF-β alone induces a regulatory cell, known as induced T regulatory cell (iTreg) (49).

Pathogens activate Toll-like receptors on APCs that induce IL-6 and IL-1β. The full maturation of Th17 cells requires IL-23 (50) and IL-21 produced by Th17 cells (51).

Th17 cells produce IL-17A and IL-17E and IL-21 and IL-22 but do not produce the Th1 cytokines IL-2, IFN-γ, or TGF-β or the Th2 cytokines IL-4, IL-5, or IL-13. The Th1 cytokine IFN-γ inhibits Th17 cells and promotes Tregs (52). Th17 cells express CCR6 to promote migration to tissue.

Th17 cells provide immunity to bacteria and fungi at epithelial and mucosal barriers. IL-17A and IL-17E recruit neutrophils. IL-22 stimulates epithelial cells to produce antimicrobial agents that destroy bacteria and fungi. Th17 cells can directly kill target cells and by release of cytokine, promote IgM production to kill pathogens. Th17 cells

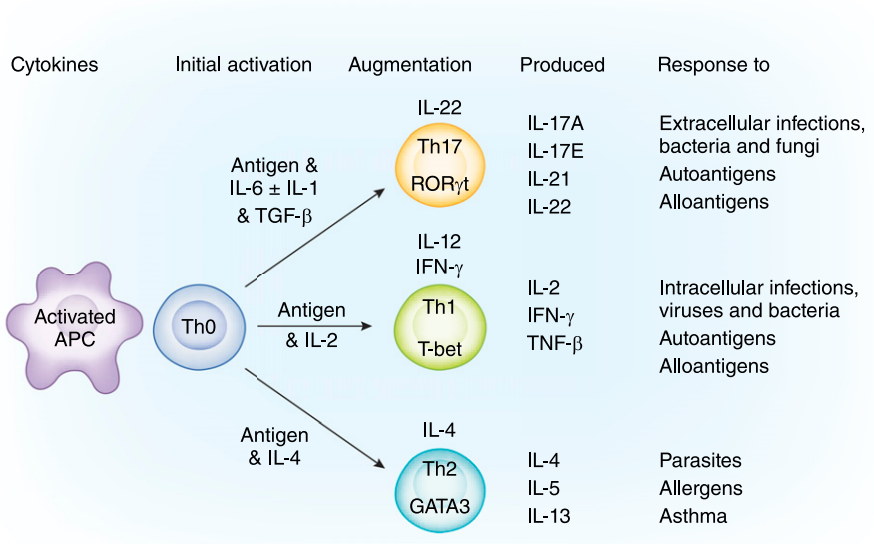

Figure 2. | **Induction of soldiers into functionally distinct cell lines.** On contact with antigen on APCs, Th0 cells can be activated to different Th subsets cells. The pathway of differentiation is driven by the nature of the antigen to which they are making an effector response. The most primitive is driven by inflammatory cytokines IL-6 and IL-1β to induce the transcription factor RORγt that produces Th17 cells producing a unique set of cytokines (top pathway). Th1 cells are initially activated by IL-2 to induce T-bet and a Th1 phenotype of cytokine expression (middle pathway). Th2 cells are induced by IL-4 to express GATA3 and Th2 cytokines (bottom pathway). The maturation of all cell lines is augmented by other cytokines: IL-22 for Th17 and IL-12 and IFN-γ for Th1. There are other lineages, such as Tfh and Th9 cells (not illustrated), that are induced by different cytokines, have a different transcription factor, and produce different cytokines.

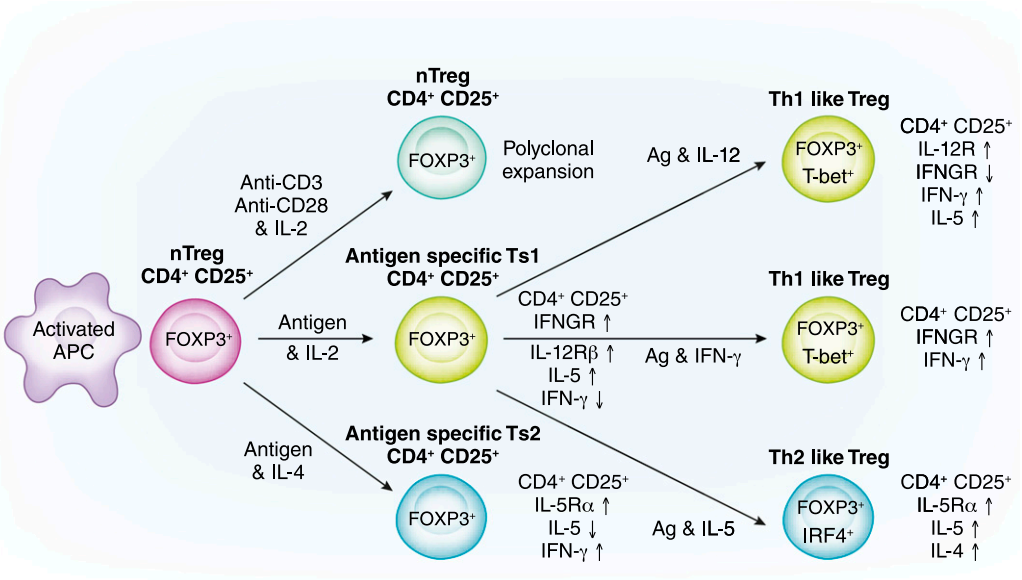

Figure 3. | **Induction of cell lines of spy cells into functionally distinct Treg lines.** The most common method of expanding natural T regulatory cells (nTregs) is polyclonal activation with anti-CD3 and anti-CD28 with high concentrations of IL-2 (top pathway). This expands nTregs that retain the nTreg phenotype and have no increased potency to suppress. In the last few years, it has been appreciated that nTregs ($CD4^+CD25^+CD127^{lo}FOXP3^+T$) are not an end line of cells that only functions to suppress the initiation of immune cells in a nonantigen-specific manner. The pathways for activation of nTregs with T cell receptors that recognize the specific antigen are as complex as those described for activation of effector T cells, which are outlined in Figure 2. With antigen and high concentrations of IL-2, nTregs within days express receptors for Th1 cytokines, IFN-γ, and IL-12 (57) (middle pathway). They have enhanced capacity to suppress to specific antigen. This is a first step in activation of these cells, which we call Ts1 cells (57). If inflammation persists, Th1 cytokines IFN-γ and IL-12 (in the absence of IL-2) can further expand the antigen-specific Treg (58). These are highly potent Th1-like Tregs that express both FOXP3 and T-bet (the Th1 transcription factor) and produce IFN-γ but not IL-2 (58). Other cytokines induce separate pathways: Th2, Th17, and Tfh responses induced Th2-, Th17-, and Tfh-like Tregs, respectively. As an example, specific antigen and IL-4, in the absence of IL-2, activate antigen-specific Ts2 cells that express receptor for IL-4 and IL-5 but not for IFN-γ and IL-12 (57) (bottom pathway). They enhance antigen-specific suppressor capacity and can be further activated by specific antigen and IL-5 in the absence of IL-4 to Th2-like Tregs that have a very potent antigen–specific suppressor capacity.

mediate many autoimmune responses (53), including multiple sclerosis, Crohn's disease, psoriasis, rheumatoid arthritis, and uveitis. Th17 also plays a role in transplant rejection and GN models (54), but their full role in renal diseases remains to be elucidated.

Th1 Cells

Th1 cells were considered the central pathway of $CD4^+$ T-cell activation for autoimmunity, intracellular infections, and allograft rejection. Activated $CD4^+$ T cells express IL-2R, a complex of α-, β-, and γ-chains that induces Jak1, Jak3, and Stat5 (55). IL-2 produced by the activated T cells acts as a growth factor inducing proliferation to Th1 cells expressing the transcription factor T-bet, which induces IFN-γ and TNF-β but not Th17 or Th2 cytokines. Th1 expresses CXCR3, which promotes migration to sites of Th1 inflammation. Th1 cells directly mediate tissue injury by release of IFN-γ and TNF-α as well as cytotoxic mechanisms, such as perforin and granzymes. Th1 cells are the principal mediators of transplant rejection and also, mediate some forms of GN (56).

Th1 cells release cytokines that induce other inflammatory cells. Th1 cytokines, IL-2, IFN-γ, and IL-12p70 help activation of $CD8^+$ T cells to cytotoxic Tc1, which expresses IFN-γ and cytolytic molecules, such as perforin and granzymes. These are cytotoxic/killer cells that destroy infected, malignant, and allografted cells. Tc1 mediates autoimmunity in type 1 diabetes and GN (56).

IFN-γ induces B cells to switch to complement-fixing Ig. Th1 cytokines IFN-γ and IL-12 activate macrophages to produce TNF-α and induce nitric oxide synthase to produce nitric oxide. These are the M1 subpopulations of macrophages that can kill bacteria and other pathogens. IFN-γ and TNF-α activate endothelial and other cells to express classes I and II MHC and ICAM1, and therefore, the T cells can interact with these cells (14).

Th1 cytokines promote antigen–specific $CD4^+CD25^+FOXP3^+$ Tregs to express receptors for Th1 cytokines IFN-γ and IL-12, which are called Ts1 cells (57), and these Ts1 cells can be activated further to Th1-like Tregs (58).

Th1 responses together with Th17 are key to targeted destruction of cells with infection or malignant transformation as well as foreign cells with alloantigen in transplants. Th1 with Th17 cells mediate many forms of autoimmunity. Th1 and Tc1 cells are key to injury in models of nephritis (56,59,60).

Th2 Cells

Th2 cells are induced in responses to parasites and allergens and are driven by IL-4 (61,62), which binds to the IL-4Rα (63) and the common γ-chain to activate Jak1 and Jak3, the transcription factors Stat4 and GATA-3 (55). $CD4^+$Th2 cells initially produce IL-4 and later, IL-5 and IL-13. Th2 cells were considered anti-inflammatory and protolerance induction (64). IFN-γ inhibits Th2 induction. Th2 expresses CCR8, which facilitates migration into tissues with Th2 inflammation.

Th2 cytokines affect other immune cells. IL-4 induces $CD8^+$ T cells to a noncytolytic Tc2 phenotype that does not express perforin, granzyme, and IFN-γ. IL-4 and IL-5 (in mice but not humans) induce Ig isotype switches in B cells to produce noncomplement-fixing IgG and IgE. IL-4 and IL-13 convert macrophages to an M2 phenotype, which lacks the inflammatory activity of M1 cells. Th2 cytokines promote antigen-specific $CD4^+CD25^+FOXP3^+$ Tregs to Ts2- and Th2-like Tregs (57,65).

Th2 responses are dominant in some forms of drug-induced interstitial nephritis, where there is eosinophilia, and can contribute to rejection. Treatment with IL-4 to promote Th2 responses reduces injury in models of nephritis (66,67) and allograft rejection (68) as does treatment with IL-5 (65,69) or IL-13 (70). These treatments reduce Th1 and macrophage activation, promote a Th2-dominant response, and induce Ts2- and Th2-like Tregs. Th2 dominance alone does not explain immune tolerance and is not necessary of tolerance induction (71,72).

Tfh Cells

Follicular helper T cells (Tfh) promote B cell maturation and activation in B cell follicles in secondary lymphoid tissues. They are induced by inducible T-cell costimulator on APCs. They function by secretion of IL-4 and IL-21 (73) and the expression of CD40L, which binds CD40 on follicular B cells and causes isotype switching of Ig, somatic hypermutation of Ig, and proliferation to germinal center B cells, thereby promoting their maturation to memory B cells and Ig-secreting plasma cells. Tfhs are regulated by the transcription factor bcl-6 (74) and express CXCR5.

Professional Spies—Tregs

The most important professional Tregs are $CD4^+CD25^+CD127^{lo}FOXP3^+$ T cells produced by the thymus. $CD4^+CD8^+$ thymocytes interact with class II MHC-expressing cells in the medullar and Hassall's corpuscles of thymus, where thymic stromal lymphopoietin promotes myeloid and plasmacytoid dendritic cells that induce Tregs (75). Treg induction requires TGF-β, stimulation of CD28 by B7.2, and IL-2 activating the IL-2R to induce Stat5 and FOXP3 (76). FOXP3 induces CD25 and inhibits IL-2 expression.

After a process of clonal deletion and selection, $CD4^+CD8^-CD25^+CD127^{lo}FOXP3^+$ T cells with a wide array of TCR specificity are released. These are naïve nTregs that are also known as thymic-derived T regulatory cells (tTregs) (77). tTregs have epigenetic demethylation of the T regulatory cell–specific demethylation region (TSDR), a promoter region of *foxp3*. This selective demethylation of TSDR makes it hard for *foxp3* to be switched off and stabilizes the nTreg lineage (78), and therefore, they and their progeny cannot revert to effector T cells (79,80). Homeostatic regulation ensures that $CD4^+CD25^+FOXP3^+$ Tregs remain as <10% of peripheral $CD4^+$ T cells (81).

nTreg survival requires low levels of IL-2, whereas effector lineage T cells express high levels of IL-7α(CD127) and depend on IL-7 to survive. Depletion of $CD127^{hi}$ cells enriches $CD4^+CD25^+FOXP3^+$ nTregs by removing activated effector lineage $CD4^+CD25^+FOXP3^-$ T cells. nTregs express helios, an ikaros transcription family member, that differentiates tTregs from periphery-induced iTregs.

Naïve nTregs express CD45RA, whereas activated effector and regulatory T cells express CD45RO. nTregs express CTLA4(CD152) and glucocorticoid-induced TNF receptor (GITR), which are also expressed by activated effector T cells. CTLA4 produced by nTregs binds B7.1(CD80) and B7.2 (CD86) to block activation through CD28, sending a negative signal to TCR/CD3 (82). nTreg through CTLA4 downregulates B7.1 and B7.2 expression by APCs (83) (Figure 1B). Fusion molecules of CTLA4 with Ig(Belatacept) prevent rejection and autoimmunity. Ipilimumab, which blocks CTLA4 and Treg, is approved for melanoma treatment (84).

nTregs at physiologic ratios of <1:10 only partially suppress naïve T-cell responses, and full suppression requires ratios of ≥1:1 to naïve $CD4^+$ T cells (85–88). Full suppression *in vivo* by nTregs requires marked depletion of effector T cells (85) or expansion of nTregs, which is transiently achieved in mice with an IL-2 and anti–IL-2 mAb complex (89). Depletion of nTregs after neonatal thymectomy (90) or anti-CD25 mAb therapy leads to autoimmunity and prevents induction of transplant tolerance.

There is a paradox, in that anti-CD25 mAb (daclizumab or basiliximab) is used as an induction therapy in patients with renal transplants and reduces rejection by depletion of activated effector $CD25^+$ T cells. Although effective in reducing rejection, these antibodies also deplete $CD25^+$ Tregs and may impede induction of transplant tolerance. The ONE Study, which is trialing immunoregulatory cells in recipients of renal transplants, excludes use of anti-CD25 mAb therapy, because it may prevent tolerance induction by host or therapeutically administered nTregs.

Activation of nTregs

CTLA4 binding to B7.1 and B7.2 blocks the activation of CD28 on nTregs, and therefore, *in vivo*, this second signal is not activated and not required for nTreg activation. The alternate second signal for nTreg activation requires high levels of IL-2 (91) compared with those required to activate effector T cells. Thus, both *in vivo* (92) and *in vitro* (93) nTreg activations are blocked by calcineurin inhibitors, which inhibit *Signal 1*. mTOR inhibitors, which inhibit *Signal 2*, block effector T-cell activation but spare Treg activation and allow preferential expansion of Tregs.

In Vitro Expansion of nTregs

Many groups propose to use nTregs to prevent graft-versus-host disease, graft rejection, and autoimmunity. To do this, they enrich nTregs and expand their number *in vitro*. The most common method for expansion of nTregs is culture with anti-CD3 and anti-CD28 mAbs with high concentrations of IL-2. Over a period of weeks, tens of thousandfold increases in nTreg numbers can be achieved. However, they remain nTregs in phenotype and function and need to be at ratios of ≥1:1 with effector lineage T cells to fully suppress an immune response. Expansion of nTreg with an mTOR inhibitor to selectively block effector T-cell activation can also be used (94). Tang and Lee (95) estimated that it will be impossible to prepare sufficient nTregs to induce tolerance for transplant or autoimmunity.

Marek-Trzonkowska *et al.* (96) in Poland were first to use nTregs as therapy in patients with type 1 diabetes and recipients of bone marrow with graft-versus-host disease (97). The ONE

Study is examining a variety of *in vitro*–activated nTregs to promote tolerance in clinical renal transplants but will be combined with conventional immunosuppression, except that anti-CD25 will not be used, because it could deplete Tregs (98). Inducing antigen-specific Tregs has also been trialed (57,58,99). The use of Tregs as a therapy was recently reviewed (100).

Activated/Antigen-Specific $CD4^+CD25^+FOXP3^+$ Tregs

There is a common misunderstanding that all $CD4^+CD25^+FOXP3^+$ T cells are nTregs. $CD4^+CD25^+FOXP3^+$ Tregs are a very heterogeneous population and include different subclasses of activated antigen-specific Tregs as well as nTregs, which have been recently reviewed (101,102). Activated antigen-specific Tregs are induced from nTregs or iTregs. The pathways for activation of antigen-specific Tregs are similar to those for activated effector T cells (Table 1).

The original examination of CD25 expression on tolerant $CD4^+$ T cells was undertaken, because tolerance-transferring cells die in culture, even if stimulated with specific antigen (15,103) but did survive if a cocktail of T cell–derived cytokines or IL-2 was present (15,103). Because IL-2 alone was insufficient to maintain antigen-specific Tregs (15,103), we examined which other cytokines promoted antigen-specific Tregs.

Culture of nTregs with alloantigen and Th1 and Th2 cytokines identified that both IL-2 and IL-4 induced polyclonal activation of nTregs (57). We identified two pathways for activation of nTregs: one by Th1 cytokines and one by Th2 cytokines (57) (Figure 3). Others have described similar pathways with Th17 and Tfh cytokines, which are reviewed in references 101 and 102. These pathways parallel those for activation of Th1 and Th2 cells and use many of the same cytokines and activation pathways as effector T cells (Table 1).

IL-2 Promotes Antigen-Specific Tregs

Numerous studies activated nTregs with antigen and IL-2 and produced antigen-specific Tregs with increased suppression to specific antigen. In our studies, culture of nTregs with specific antigen and IL-2 induces antigen-specific Tregs that express the receptors for IFN-γ (57) and IL-12 (58) (Figure 3). These $CD4^+CD25^+$ T cells express FOXP3 and IL-5 but not IFN-γ or IL-2, and we named them Ts1 cells (57). They have increased antigen–specific suppressor potency *in vivo* and *in vitro*, suppressing at $<$1:10, whereas fresh nTregs only fully suppress at $\geq$1:1.

Additional activation of these cells with antigen and IL-12 without IL-2 induces a more potent antigen-specific Th1-like Treg (58) (Figure 3, Table 1). The Th1-like Tregs that we generated suppressed *in vitro* at 1:1000 and delayed fully allogeneic graft rejection in nonimmunosuppressed normal hosts (58). These Th1-like Tregs expressed FOXP3, T-bet, and the receptors for IL-12 and IFN-γ. They expressed IFN-γ but not IL-2. They are considered Th1-like Tregs, because they express T-bet and IFN-γ. Of note, the continued presence of IL-2 blocks generation of Th1-like Tregs, suggesting that the current methods of Treg expansion with repeated IL-2 exposure may select against growth of antigen-specific activated Tregs (58). Th1-like Tregs occur in humans, including recipients of renal transplants (104).

Th1-like Tregs can be generated by IL-12 (105,106), IFN-γ (107,108), or IL-27, but the final Treg induced by each pathway is probably different.

IL-4 Promotes Antigen-Specific Tregs

In our studies, culture of nTregs with specific antigen and IL-4 induces antigen-specific Tregs that express the specific IL-5 receptor (IL-5Rα CD125) (109). These $CD4^+CD25^+$ T cells express FOXP3 and IFN-γ but not IL-5 or IL-2 (57,65) and were named Ts2 cells. Ts2 cells have increased antigen–specific suppressor potency *in vivo* and *in vitro*, suppressing at $<$1:10, whereas fresh nTregs only fully suppress at $\geq$1:1. We found that treatment with IL-5 promoted these cells to control autoimmunity (65) and transplant rejection (57). Tregs controlling Th2 responses express the transcription factor IRF4 (110). Additional activation of these cells with antigen and IL-5 and without IL-4 induces a more potent antigen-specific Th2-like Treg (Figure 3).

Soldiers Become Spies

In Situations with No Inflammation

T effector lineage cells contacting antigen are not activated or converted to iTregs (Figure 4). Naïve $CD4^+CD25^-$ T cells that contact an antigen that their TCR recognizes can convert to an antigen-specific iTreg if there is TGF-β but no IL-6. Thus, in normal tissue remodeling or after noninflammatory tissue injury, the autoantigens released do not activate effector T cells, because there are no inflammatory cytokines. TGF-β produced to promote tissue repair induces protective iTregs to prevent autoimmunity.

Anergy

Effector lineage T cells that contact specific antigens through their TCR/CD3 and other ligands associated with *Signal 1* that do not receive a second signal through CD28 become anergic (27). Anergic cells are not activated to proliferate or express IL-2 if re-exposed to the specific antigen with a *Signal 2*. They cannot be mobilized as a soldier.

Th3 Cells

The first $CD4^+$ Tregs that were described to be induced from effector lineages were Th3. Th3 cells are induced in mucosa by specialized dendritic antigen presenting cells, known as $CD103^+$DC (111). Th3 is suppressed in mucosa by release of IL-10 and TGF-β (112). Th3 cells induce oral tolerance induced by antigen exposure through the gut.

iTregs

$CD4^+CD25^-FOXP3^-$ T cells that are exposed to antigen in the presence of TGF-β (49), where there is no IL-6 or IL-1β by inflammation, are induced to express FOXP3 (113,114). TGF-β inhibits RORγT expression and development of Th17 cells (115). These iTregs have a $CD4^+CD25^+$ phenotype and express other markers of nTregs, such as CTLA4 and GITR, but they do not have demethylation of TSDR of FOXP3 and do not express helios. Thus, expression of FOXP3 is not stable (78). This process of generation of iTregs increases the number of antigen-specific Tregs when there is autoantigen released by normal tissue remodeling and noninflammatory tissue injury (101,102) that is associated with TGF-β release (116).

iTregs can control Th17 responses in autoimmunity (114) and rescue scurfy mice (113). iTreg induction is inhibited by the presence of IL-6 (117). The presence of IL-4 with TGF-β induces Th9 cells expressing IL-9 and IL-10 (118).

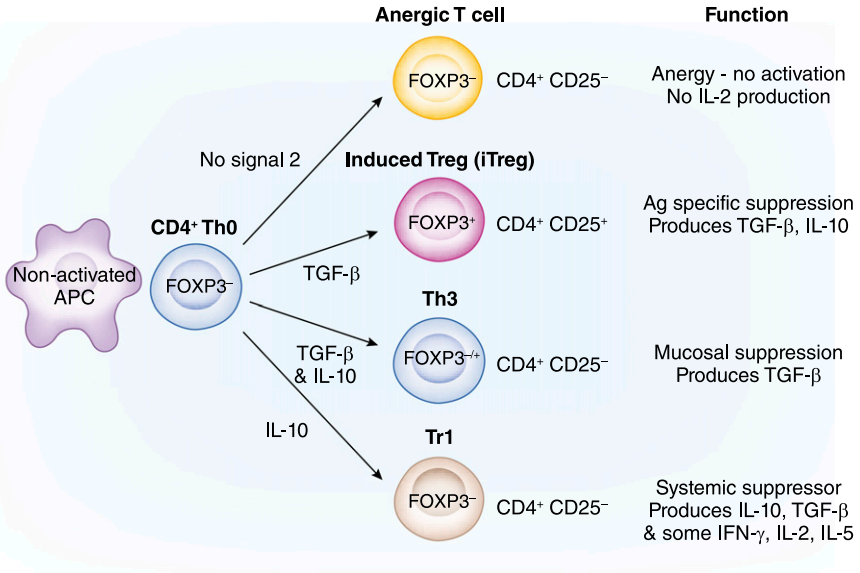

Figure 4. | **Newly recruited CD4$^+$ T-cell soldiers induced to be spies.** Professional soldier lineage CD4$^+$CD2$^-$CD127^{hi}FOXP3$^-$ T cells can fail to be activated into effector cells when they contact antigen. This figure illustrates four pathways that will be described in order from top to bottom. (*1*) Anergy is induced when there is no *Signal 2*, and these cells, when re-exposed to specific antigen with activated antigen-presenting cells (APCs), are not activated and do not proliferate or produce IL-2. This occurs in the absence of inflammation and activated APCs (pathway 1). (*2*) Induced T regulatory cells (iTregs) are induced by TGF-β and antigen when there is no IL-6 or IL-1β (pathway 2). These cells express FOXP3 and CD25 but can revert to effector T cells, because FOXP3 expressions are not stable. (*3*) In the mucosa, to induce oral tolerance, TGF-β and antigen can induce Th3 cells that can express FOXP3 (pathway 3). Th3 cells suppress by release of TGF-β. (*4*) Tr1 cells are induced by repeated culture with antigen and IL-10, which converts the APCs to DC-10 cells (pathway 4). They do not express FOXP3 or CD25 and suppress by release of TGF-β and IL-10.

iTreg survival depends on IL-2 (119,120); iTregs can revert to effector T cells (121).

Tr1 Cells

Tr1 cells are induced by repeated culture of naïve CD4$^+$ T cells with antigen and IL-10, which induces APCs to DC-10 cells (122). Tr1 cells are CD4$^+$CD25$^-$Foxp3$^-$ T cells that produce IL-10 and TGF-β as well as some IL-5, IFN-γ, and IL-2 but no IL-4 (123). Tr1 cells suppress autoimmune and allograft responses by release of IL-10 and TGF-β and through perforin/granzyme B (124). Therapy with Tr1 cells is in clinical trials.

Activated Effector T Cells Fail to Fight or Become Spies

T Effectors Die from Exhaustion

T effectors die from exhaustion from ongoing activation and proliferation, leading to clonal pruning with a reduction in the number of antigen-reactive clones (125) (Figure 5). The mechanism of clonal exhaustion remains unclear but is driven by persistent antigen activation of TCRs (126). It may include Treg elimination of effector T cells.

T Effectors Die of Activation-Induced Cell Death

Activation-induced cell death can be caused by Fas/FasL-mediated apoptosis after repeated stimulation of TCR inducing FasL (127). Fas/FasL also induced apoptosis in Tregs (128). The Fas/FasL pathway alone cannot induce immune tolerance (129).

Activated T cells as well as B cells and macrophages express programmed cell death protein-1 (PD1; CD279), a member of CD28 family. PD1 on binding to programmed cell death protein-1 ligand (PD-L1) or PD-L1/B7 complex blocks TCR signaling (130) and can lead to activated T-cell deaths. PD-L1 in normal tissue is expressed in kidney, heart, lung, thymus, and spleen and upregulated on dendritic cells and macrophages during inflammation. The second ligand for PD1 is PD-L2 that is restricted to dendritic and tumor cells.

PD1 knockout mice develop lupus nephritis and cardiomyopathy, suggesting that this pathway prevents autoimmunity in the kidney and heart. PD-L1 is expressed on many tumor cells, and treatment with mAbs against PD1 (nivolumab and pembrolizumab) is effective in some patients with melanoma, nonsmall cell lung cancer, or renal cell cancer. Treatment with PD1 antagonists can unmask autoimmunity and theoretically, may unmask rejection of renal transplants as may inhibitors of CTLA4.

Activated effector T cells during intense inflammation are induced to express IL-10 (131). IL-27 binds to the IL-27 receptor (132) on Th1, Th2, or Th17 cells and induces IL-10 (133,134). IL-10 is anti-inflammatory and prevents APC activation. IL-27 is a member of the IL-12 family, in which cytokines and their receptors are heterodimers formed by various combinations of proteins in the family (135). IL-12, IL-23, and IL-27 promote effector T cells and induce IFN-γ. IL-12p40, IL-27, and IL-35 inhibit activated T cells. IL-12 (58,105,106) and IL-27 promote Tregs to Th1-like Tregs (105).

Activated Tregs infect activated T cells to become Tregs by two mechanisms (43,136). First, TGF-β on the surface of

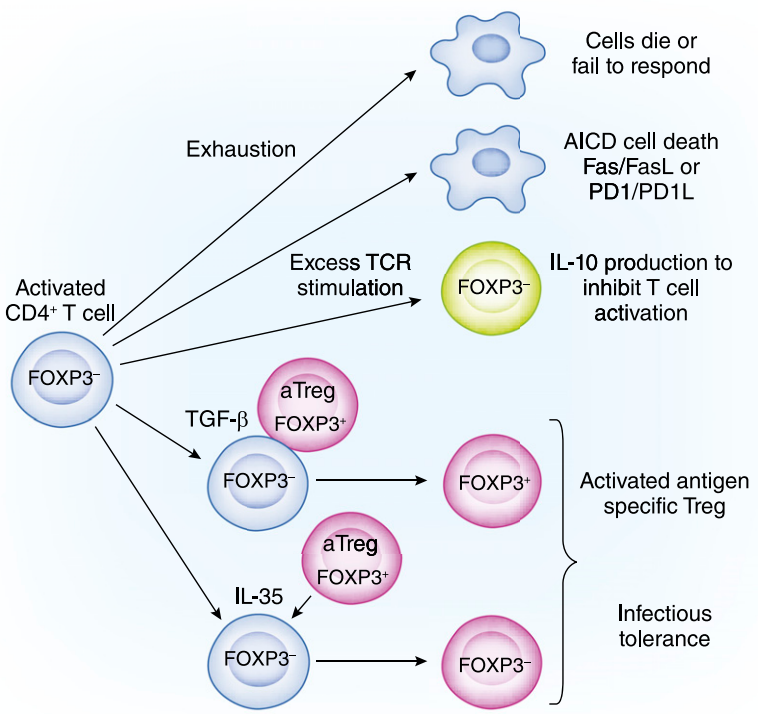

Figure 5. | **Activated and aggressive CD4$^+$ T cell soldiers convert to spies**. Fully activated T cells programmed to mediate antigen-specific injury can be neutralized or change sides. Five pathways are illustrated, and described from top to bottom. (*1*) Neutralization or cell death occurs from repeated T cell receptor (TCR) activation and proliferation of effector T cells (pathway 1). The cells can die of exhaustion, leading to clonal pruning. (*2*) Excessive and repeated stimulation of TCRs on effector cells induces them to express surface molecules that, when they bind ligand, induces apoptosis (pathway 2). This leads to activation induced cell death (AICD). The best described pathways are Fas/FasL and programmed cell death protein-1 (PD1) /programmed cell death protein-1 ligand (PD-L1). (*3*) Induction of IL-10 expression by effector T cells (pathway 3). After repeated stimulation and expansion, activated effector T cells can be induced to express IL-10 usually by IL-27 binding to IL-27R. The release of IL-10 by these effector T cells reduces inflammation, especially the activation of APCs. The last two pathways involve T regulatory cells (Tregs) infection of effector T cells to convert them to Tregs. Direct contact with activated Tregs can infect effector T cells to make them regulatory. (*4*) TGF-β on Treg surface can, by cell-cell contact, induce FOXP3 and endow Treg function in effector T cells (pathway 4). (*5*) IL-35 released from activated Tregs binds the IL-35 receptor on activated T cells and converts them into FOXP3$^-$ iTr35 cells (pathway 5). AICD, activation induced cell death.

Tregs binds to activated T effectors by a TGF-β receptor and induces expression of FOXP3 and the ability to suppress (137). Second, activated Tregs secrete IL-35 that binds to IL-35 receptor on activated effector T cells and converts them to iTr35. iTr35s are distinct from iTregs and Tr1 cells and are FOXP3$^-$ (138). iTr35s occur in humans and are potent suppressors (138).

Mechanisms of Action of Activated Tregs

A variety of mechanisms mediates suppression. With nTregs, CTLA4 binds to B7.1 and B7.2 to block these molecules and prevent T-cell activation. It is an oversimplification to attribute all suppression to IL-10 and TGF-β, which mainly suppress in mucosa (139).

With activated Tregs, IL-10 or IL-35 can suppress but is not essential (140). Activated Tregs can express CD39 and CD73 that metabolize extracellular ATP and ADP to adenosine, which suppresses activated effector T cells through the A2A adenosine receptor (141). IFN-γ, perforin, and granzyme B used by cytotoxic T cells also mediate suppression by some activated Tregs (142,143); thus, the main weapons of cytotoxic T cells are used by Tregs to suppress.

Activated Tregs can suppress the function of activated CD4$^+$ T cells, CD8$^+$ T cells, B cells, and macrophages. They control all aspects of immunity, albeit that memory CD4$^+$ T cells are less responsive to control by Tregs (15).

Do Spies Become Soldiers?

There is concern that, if Tregs can change to effector lineage, their use as therapy may be unreliable, if not dangerous (144–147). At present, the consensus is that nTregs/tTregs that have demethylation of TSDR are stable, and their progeny remain Tregs (144,145). nTregs have demethylation of regions of other genes essential to their function, including CTLA4 and GITR (148). Transfer of nTregs to lymphopenic hosts, where there is inadequate IL-2, can lead to transient loss of FOXP3 (149). In uncontrolled immune inflammation, Tregs can be induced to the Th1-, Th2-, or Th17-like Tregs as described above. Whether these cells are effector or only suppressor is not resolved, but to survive, they depend on cytokines produced by the effector T cells (57,58,103).

Induced or peripherally generated Tregs (iTregs/pTregs) that develop from effector lineage T cells activated by antigen and TGF-β if there is no IL-6 or IL-1 express FOXP3 and become regulatory. These cells are plastic and readily

revert to effector lineage if exposed to IL-6 in the absence of TGF-β (150).

Role of T Cells in Renal Diseases

Although antibodies are considered the main mediators of GN, T cells also play a central role. First, T cells provide help for isotype switching of antibody from IgM to IgG, IgA, and IgE. Th1 cells provide the cytokines to promote development of complement fixing antibodies, such as IgG1 and IgG3. Th2 cells promote noncomplement fixing antibodies IgG2 and IgG4. IgA induction requires TGF-β from T cells in the mucosa. Thf cells promote B-cell proliferation and the maturation of B-cell response in lymphoid follicles.

There is compelling evidence that T cells also contribute directly to glomerular injury (151), especially in nephritis, where there is no or little Ig and complement deposition. In experimental models, infiltration of T cells in glomeruli is associated with injury, especially Th1 and Th17 cells (54,60,66) but not Th2 cells, which tend to be protective (67,152). These are reviewed in an companion article by Holdsworth and Gan (153). Although Tregs can suppress nephritis in animal models (154), the potential of these cells as a therapy requires more investigation.

In drug-induced interstitial nephritis, there are Th2 and Th1 responses.

There is a T cell and macrophage interstitial infiltrate in the kidney in acute ischemia (155), with ureteric obstruction, and in many forms of GN and end stage renal failure. In AKI, Th1 responses are present (156), and injury can be reduced by Treg (157) depletion of T cells (158) and blocking T-cell migration into kidneys (159). Whether T cells contribute to injury or are a benign reaction (160) remains to be resolved.

Role of T Cells in Transplant Rejection

Acute cellular rejection is T cell–mediated (30,161) and involves $CD4^+$ and $CD8^+$ T cells (162). The $CD4^+$ T cells mediating rejection are Th1 (163) and Th17 but can include Th2 cells (72). Alloantibody responses are also dependent on help from $CD4^+$ T cells. Transplant tolerance is mediated by $CD4^+$ T cells (15,164), and $CD4^+CD25^+$ T cells (15) are essential for induction and maintenance of tolerance. nTregs and alloantigen-activated Tregs are being trialed as therapy to reduce rejection with an ultimate aim of inducing tolerance (98).

There is much overlap in the molecules and pathways used by T cells that act as soldiers and spies. There is also considerable plasticity in that soldiers can fail to fight or become active spies. The presence of spies has benefits in controlling unwanted destructive immune response damaging vital tissues, such as the kidney. Inadequate Treg responses can lead to autoimmunity. Activation of Tregs induces tolerance to allografts, bone marrow grafts, and normal host tissues in autoimmunity. Destroying the spies may allow immune destruction of tumor cells. How to control T cell–mediated injury is of relevance to nephrologists caring for patients with renal transplants and immune-mediated diseases, such as GN, interstitial nephritis, and possibly, AKI.

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Cytokines: Names and Numbers You Should Care About

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Abstract

Cytokines play an important role in host defense against microorganisms. They orchestrate innate immunity by inducing protective local inflammation and systemic acute phase responses. Cytokines are important in initiating, amplifying, directing, mediating, and regulating adaptive immunity. Unfortunately, they may also direct tissue damage if excessive responses occur or if they are involved in directing and mediating autoimmunity. Under these circumstances, cytokines are potential therapeutic targets. Over the last 20 years, we have seen the successful development and clinical implementation of biologic strategies that target key cytokines in specific inflammatory diseases with efficacy, specificity, and toxicity profiles challenging conventional drug therapies. These therapies are finding new applications and many new agents show promise. Unfortunately, these new cytokine-based therapies have had little effect on renal disease. This review provides evidence that common renal diseases, including those causing AKI and the autoimmune proliferative and crescentic forms of GN, have cytokine mediation profiles that suggest they would be susceptible to cytokine-targeting therapeutic strategies.

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Introduction

Cytokines amplify and direct the generation of appropriate patterns of immunity to combat particular microbial threats. These same cytokines can cause host tissue injury if the activation/amplification of host defense is overexuberant, as occurs in some infective and sterile forms of inflammation. These pathologic cytokine-driven outcomes are seen in many types of AKI caused by physical, drug, chemical, and ischemic injury. If immune tolerance is lost and host tissue antigens become autoimmune targets, cytokines can direct and mediate inflammatory autoimmune diseases. Important renal examples are the autoimmune forms of inflammatory/crescentic GN. Cytokines act in concert to generate inflammation in host defense and disease, but some cytokines attenuate inflammation and induce repair. Individual cytokines can be inhibited by antibodies or competitive receptors or by the therapeutic use of immunomodulatory cytokines. These biologic agents are now widely used to treat inflammatory diseases. There are a number of common renal diseases that could potentially be treated by targeting cytokines. This review will address these issues.

Cytokine Nomenclature

Cytokines are glycoproteins that regulate the functions of the immune system. Definitions are imprecise because of redundancy of function and the capacity of tissue parenchymal cells and leukocytes to produce them. Hence the terms lymphokine and monokine have been dropped. Originally described by their perceived major function, the term IL has been adopted. When an agreed characterization of a cytokine is broadly accepted, a number is attributed (*e.g.*, IL-6). However, the use of descriptive names for some key cytokines persists, including IFNs (α, β, and γ), TNF (TNF-α and TNF-β), colony stimulating factors (granulocyte colony–stimulating factor [G-CSF] and granulocyte–macrophage colony–stimulating factor [GM-CSF]), and some growth factors (TGF-β and PDGF).

Cytokines in Host Defense

Innate Immunity

Most living organisms rely on innate immunity (in the absence of adaptive immunity) for host defense. Cytokines play critical roles in orchestrating the rapid effective response of leukocytes and parenchymal cells to the detection of microorganisms or significant noninfective damage to parenchymal cells. These cells are hardwired with receptors that recognize and respond to common pathogen proteins through Toll-like receptors (TLRs) and danger-associated molecular pattern receptors (1). Although leukocytes are the major source of innate cytokine responses, parenchymal cells are increasingly recognized as also producing innate inflammatory cytokines and interacting with leukocytes to optimize cytokine responses in generating host defense. The major acute innate cytokines, IL-1, TNF-α, IL-6, IL-12, CXCL8 (formerly IL-18), G-CSF, and GM-CSF, are used locally to activate endothelial cells and local tissue leukocytes (mast cells [MCs], dendritic cells [DCs], $\gamma\delta$ T cells, and neurones), triggering cytokine-mediated amplification loops generating chemokine release, generating endothelial cell adhesion molecule expression, slowing blood flow, and increasing vascular permeability. These changes facilitate the accumulation of humoral defense proteins, complement, coagulation proteins, acute phase proteins, and Ig. They also recruit and activate a range of leukocytes (including innate lymphocytes, $\gamma\delta$ T cells, natural killer cells, natural killer T cells [NKT] cells, and innate

leukocyte-like cells). The net result of these orchestrated events is inflammation. Concurrently, two further processes are initiated: a cohort of cytokines (IL-1, IL-6, and TNF-α) are generated that act systemically to prepare the whole organism for microbial defense by initiating the acute response syndrome (2) and local foreign material is processed and presented by antigen-presenting cells (APCs) to initiate adaptive immunity. These secondary processes are also critically dependent on cytokine direction and are also responsive to inhibitory cytokine modulation (Figure 1).

Adaptive Immunity

CD4+ T cells play a central role in adaptive immunity which is characterized by antigen specificity and memory/recall capacity. T-cell specificity is a consequence of the diversity of T cell receptors (TCRs) from which thymic processing eliminates potentially autoreactive T cells to form the T-cell repertoire. Antigen recognition (signal 1) is initiated by TCR binding to antigens ingested, processed, and presented (as peptides) on major histocompatibility complex molecules by specialized APCs. Activation of T cells requires secondary signals provided by costimulatory molecules (including CD40, CD80, and CD86) expressed on APCs to engage CD154 or CD28 on T cells. Innate cytokines released at the time of antigen presentation (a third signal) determine the specific Th subset differentiation pathway the (now activated) T cells will follow. In 1986, Mossman and Coffman demonstrated that there were two separate pathways of Th subset differentiation, Th1 and Th2 (3). Th1 lineage commitment is directed by IL-12, which induces the specific signal transducers and activators of transcription (STAT) factors, STAT4 and T-bet, resulting in the production of effector cytokines, IFN-γ, and TNF-α by the differentiated Th1 cells. Th1 cells activate macrophages to mount cell-mediated immune responses against intracellular pathogens. Th2 differentiation occurs in the presence of IL-4 to activate transcription factors STAT6 and GATA3 producing IL-4, IL-5, IL-9, and IL-13 that drives humoral and IgE mediated immunity. In 2005, a new distinct Th subset was defined on the basis of its predominant production of IL-17, named Th17 cells (4). Lineage commitment of Th17 cells requires TGF-β and IL-6 for the expression of the transcription factors, STAT3 and RORγ-t, whereas maintenance and expansion of Th17 cells relies on IL-23. Activated Th17 cells produce IL-17A-F, IL-21, IL-23, and IL-22 and activate cells, particularly neutrophils, important in host defense against extracellular pathogens. Antigen presentation by TGF-β alone activates Foxp3-inducing regulatory T cells (Tregs), producing IL-10, TGF-β, and IL-35. Tregs play an important role in modulating effector T-cell responses and preventing autoimmunity.

Three further Th subsets have been defined: Th9, Th22, and T follicular helper. The Th2 subset is reprogrammed to become Th9 when IL-4 plus TGF-β activates STAT6, IRF4, GATA3, and PU.1 to produce IL-9, IL-10, IL-17, IL-21, and IL-22, promoting tissue inflammation (5). Th22 is important in skin immunity (protection and regeneration). TGF-α and IL-6 activates transcription factor, AHR, to direct the differentiation of IL-22, producing Th22 cells. T follicular helper cells migrate to follicular B-cell zones via CXCR5 where they help B cells activating Bcl-6 to produce IL-21 (6) (Table 1).

Cytokines as Therapeutic Targets

Many conventional immunomodulating drugs induce their therapeutic effects, at least in part, by attenuating the actions of injurious cytokines. These drugs include glucocorticoids. Their targets include transcription factors, nuclear factor-κ B, and activator protein 1, inducing transcription of inflammatory cytokines (7). They also effect post-translational events, including intracellular signaling and effector cytokine mRNA stability (8). Downstream effects reduce leukocyte trafficking (by attenuating TNF-α– and IL-1β–enhanced endothelial cell

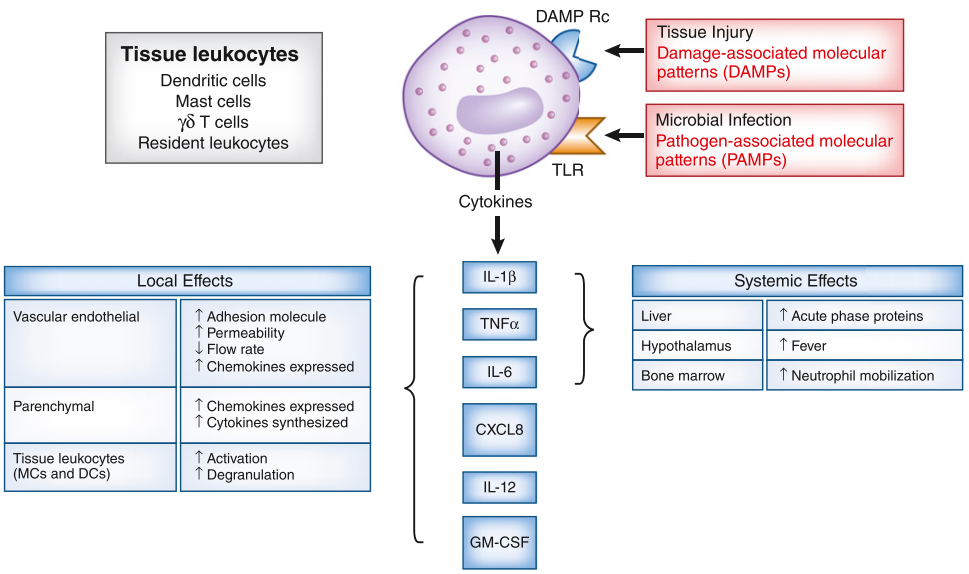

Figure 1. | Production of major acute innate cytokines involved in local and systemic responses following leukocyte activation via TLRs or danger-associated molecular pattern receptors. DC, dendritic cell; GM-CSF, granulocyte-macrophage colony–stimulating factor; MC, mast cell; Rc, receptor; TLR, Toll-like receptor.

Table 1. Phenotypical and functional characteristic profiling of CD4+ T helper subsets

Characteristic	Treg	Th1	Th2	Th17	Th9	Th22	TFH
Pathway determining cytokines	IL-10	IL-12, IFN-γ	IL-4	TGF-β, IL-6, IL-23	TGF-β, IL-4	TGF-α, IL-6	IL-21, IL-6
Cell surface chemokine receptors	CCR7, CXCR4	CXCR3, CCR5	CCR3, CCR4, CCR8, CXCR4	CCR4, CCR6	—	CCR4, CCR6, CCR10	CXCR4, CXCR5
Transcription factors	Foxp3	STAT4, T-bet	STAT6, GATA3	STAT3, RORγt	STAT6, IRF4, GATA3	Arh	Bcl-6
Signature cytokines	IL-10, TGF-β, IL-35	IFN-γ, TNF-α	IL-4, IL-5, IL-13	IL-17A–F, IL-21, IL-22, IL-23	IL-9, IL-10, IL-17, IL-21, IL-22	IL-22	IL-21
Effector responses	Immune tolerance	Cell mediated immunity	Humoral and IgE immunity	Autoimmunity	Tissue inflammation and mucus production	Skin homeostasis and pathology	B cell maturation

Treg, regulatory T cells; TFH, T follicular helper.

adhesion molecule expression). They reduce the number of circulating T cells and inhibit IL-2 production. Th cell differentiation shows a shift to Th2 with attenuation of monocyte IL-12 without effecting IL-10 production. This results in the reduction of Th1 responses favoring Th17 (9). A number of other well established anti-inflammatory drugs (pentoxifylline and thalidomide) also attenuate inflammatory cytokine gene transcription (10).

Biologic Approaches to Cytokine Therapeutic Manipulation

Attempts to biologically target cytokines were led by studies in shock; however, their beneficial effects were limited. Clinical trials in rheumatoid arthritis (RA) and psoriasis were more effective. These studies in RA and psoriasis were facilitated by the capacity to repeatedly access the affected tissues (synovial joints and skin) to determine dominant cytokines and to correlate their presence with disease severity, outcomes, and treatment responses. Administering immunoneutralizing candidate cytokines in synovial and dermal tissues *in vitro* allows their biologic effect to be studied. Finally, preclinical study of the biologic effects of administering or blocking cytokines *in vivo* in relevant animal models provided proof of concept for efficacy, specificity, and potential toxicities. The clinical success of anti–TNF-α monoclonal antibodies (mAbs) in human RA and psoriasis helped establish an accepted role for these biologics as mainstream therapeutics (11). Subsequently, anti–TNF-α strategies were successfully applied to other related rheumatologic diseases (12) and then to inflammatory bowel disease (IBD) (13). However, anti–TNF-α therapy was not effective in ANCA vasculitis (14) and multiple sclerosis (MS) (15). A number of different strategies have been used to achieve therapeutic outcomes by cytokine manipulation. Most involve neutralizing cytokine effects in disease either by immunoneutralization or the use of competitive decoy receptors (Table 2). Several potentially therapeutic mAbs targeting other key innate proinflammatory cytokines (particularly IL-1 and IL-6) were developed and tested in a number of inflammatory and autoimmune diseases.

Cytokines for Which Biologic Therapies Have Shown Clinical Effectiveness

TNF-α

There are now five approved therapeutics for rheumatologic use, including RA, ankylosing spondylitis, and psoriatic arthritis. Four are mAbs (infliximab, centolizumab, adalimumab, and golimumab), whereas etanercept is a recombinant human TNF receptor (Rc) fused to the Fc portion of IgG that acts as a competitive inhibitor. These therapeutics modify inflammatory joint damage and systemic inflammatory symptoms (16). Optimal outcomes in RA often require combination of conventional methotrexate with anti–TNF-α mAb (17).

IL-6

IL-6 is a pleiotropic cytokine first described as a T- and B-cell growth factor produced by T cells, macrophages, and endothelial cells. It is a potent inducer of local and systemic inflammation where it plays a key role in the acute phase response (*e.g.*, IL-6 binds to cell surface IL-6Rc and

Table 2. Strategies used for therapeutic cytokine manipulation

Monoclonal antibody-mediated cytokine immunoneutralization	IL-1, IL-6, TNF-α, IL-12, IL-23/IL-23, IL-17, IFN-α
Modified cytokine receptors: competitive inhibitors	CTLA-4, TNF-α Rc
Upstream drugs with efficacy through cytokine attenuation	Syk, S1P, BLyS, JAK
Immunomodulating cytokines as therapeutic agents	IL-10, TGF-β
Cytokine gene therapy	IL-1Ra

CTLA-4, cytotoxic T lymphocyte antigen-4; Rc, receptor; Syk, spleen tyrosine kinase; BLyS, B lymphocyte stimulator; JAK, Janus kinase.

signaling is facilitated by glycoprotein 130). Tocilizumab is a mAb targeting IL-6Ra. This mAb attenuates joint inflammation, bone erosion, and systemic inflammation in RA (18). Tocilizumab is also effective in juvenile RA (Still's disease) and Castleman's disease (19).

IL-1

IL-1 is an innate cytokine with powerful capacity to activate macrophages and epithelial cells and acts in concert with IL-6 to induce systemic acute phase responses. It has a natural antagonist, IL-1RA. IL-1RA competitively inhibits IL-1Rc binding by IL-1α and IL-1β. Anakinra is a human recombinant form of IL-1RA. Therapeutically, anakinra has been unsuccessful compared with anti–TNF-α therapy for RA, but it is highly effective in modulating the Cryopyrin-associated periodic syndromes, including neonatal onset multisystem inflammatory disease, Muckle–Wells syndrome, acute and chronic gout, and juvenile RA (20).

IL-2

IL-2 is a growth factor for activated T cells. CD28-dependent costimulation of activated T cells induces expression of the high affinity IL-2 (γ, β, and δ) receptor (CD25). Basiliximab is a mAb designed to bind and block the IL-2Rc on activated T cells. This mAb is widely used to prevent early kidney transplant rejection.

A Cochrane systematic review shows that basiliximab is effective at reducing rejection 3 and 6 months postrenal transplantation. Because Tregs express high levels of CD25, there is the potential risk that its efficacy may be limited by its potential effect on blocking immunomodulation. A humanized anti–IL-2Rc mAb, daclizumab shows no apparent differences from basiliximab (21) and has been reported to be effective in treating uveitis in eight of ten patients in an open-label study (22).

IL-12/IL-23

These dimeric molecules share one chain in common, p40. Targeting p40 offers the opportunity to attenuate both Th1 (driven by IL-12) and Th17 (enhanced by IL-23) pathways of Th differentiation. Ustekinumab and briakinumab are such inhibitory mAbs. They have been assessed in severe refractory Crohn's disease but without efficacy (23). Ustekinumab is also effective in psoriasis (24).

IL-17A

IL-17A is important in host defense by mobilizing and activating neutrophils, whereas pathologic IL-17A responses lead to the development of autoimmunity. Secukinumb is an inhibitory anti–IL-17A mAb and is effective in psoriasis, psoriatic arthritis, and ankylosing spondylitis (25); however, studies in RA show mixed results and no clear consensus of significant benefit (26). Furthermore, in Crohn's disease treated with secukinumb, no benefit or disease excerbation was observed (27). In a phase II clinical trial, secukinumab treatment showed promising results in MS (28).

IL-10

This cytokine can attenuate the production of inflammatory cytokines. IL-10 is a prominent participant in human inflammatory diseases (*e.g.*, significant amounts can be measured in the synovium of patients with RA). Administration of IL-10 did not attenuate RA activity (29), but it is beneficial in psoriatic arthritis (30). In ulcerative colitis, IL-10 was ineffective when administered at doses that were not associated with side effects, including anemia (31).

Biologic Therapies that Have Downstream Anticytokine Effects

Cytotoxic T Lymphocyte Antigen-4

While strictly speaking, cytotoxic T lymphocyte antigen-4 (CTLA-4) Ig is not primarily a cytokine therapy, blockade of costimulatory molecules essential to adaptive immunity can secondarily block cytokine-mediated inflammation. T-cell surface receptors CD28 and CTLA-4 bind APC ligands CD80 and CD86. CD28 is pivotal in enhancing immune activation, whereas CTLA-4 delivers an inhibitory signal. Abatacept and belatecept are approved by the US Food and Drug Administration for treatment of resistant RA (32). It is effective in this situation; however, it as expected carries a risk of serious infection.

Cytokine Gene Therapy

Gene therapy has been demonstrated to be an effective way of treating pathologic inflammation. Rheumatoid synovia are arthroscopically removed from patients awaiting joint replacement and transfected with the gene for IL-1RA. Reimplantation of this transfected synovia back into the joint attenuated disease (33). This technique has also been successfully used in collagen arthritis.

Cytokine Signal Transduction Inhibition

Blocking cytokine signaling pathways can effectively prevent cytokine participation in inflammatory diseases. A number of small molecular weight inhibitors have progressed to clinical trial; however, specificity and toxicity have limited their progress to the clinic.

Janus Kinase Inhibitors

Janus kinase (JAK) inhibitors are small molecules with multiple effects on cytokine signaling pathways that inhibit

the effects of cytokine-induced cell activation and consequent pathologic inflammation (34). Tofacitinib preferentially inhibits JAK-1 and JAK-3. In clinical trials, the degree of benefit in resistant RA was similar in efficacy to adalimumab, a TNF-α inhibitor. It may lack specificity because side effects, including sepsis, disturbed liver function tests, raised creatinine, and neutropenia, were reported during its use (35).

Spleen Kinase Inhibitors

Inhibition of spleen kinase signal transduction pathway prevents downstream gene transcription (*i.e.,* synthesis of proinflammatory cytokines). The best studied spleen kinase inhibitor is fostamatinib. It has been successfully used in collagen arthritis in mice (36) and has been studied in >3000 patients with RA in several clinical trials. Responder rates for acute disease were encouraging, but side effects were common (37). Fostamatinib acts by inhibiting TNF-α–induced IL-6 production by fibroblast-like synoviocytes (38).

Collectively, data from current clinical trials are insufficient to draw general statements about cytokine therapeutic manipulation in chronic autoimmune inflammatory disease in humans. However, several observations seem appropriate. TNF-α is clearly an important mediator in injurious inflammation in several autoimmune and autoinflammatory diseases. However, there is also evidence to suggest that in other diseases, it is either redundant or potentially immunomodulatory (MS and ANCA vasculitis). IL-1β is also strongly linked to most autoinflammatory diseases and gout. Clinical trials with IL-6 are more limited, it is present together with TNF-α (in RA) and both TNF-α and IL-1β in autoinflammatory diseases. The fact that individual immunoneutralization of IL-6 is beneficial in these diseases suggests independent requirement for the expression of this cytokine. The effectiveness of IL-17 and IL-23 immunoneutralization to attenuate psoriasis and psoriatic arthritis supports the case for these diseases being Th17 mediated. However, TNF-α is also required for the generation of inflammation in this disease because TNF-α neutralization is also beneficial. Although experimental animal models have provided evidence that the CD4+ Th17 subset directs inflammatory injury in RA and IBD, the clinical data available do not support this role in human RA and IBD. However, preliminary data in MS, targeting IL-17, shows evidence of benefit. Although much more data are necessary before firm conclusions can be drawn, these observations on the therapeutic benefit on inhibiting single cytokines suggest that different combinations of cytokine may direct specific patterns of disease (Table 3).

Therapeutic Cytokine Targeting in Renal Diseases

There are three renal diseases where there is good evidence that immune cytokines play significant roles in disease pathogenesis and where their therapeutic manipulation could potentially be efficacious. These diseases are AKI, lupus nephritis, and proliferative/crescentic GN.

Innate Immunity: AKI

AKI is the most common hospital-based kidney disease (37). Much of our understanding of the mechanisms of injury in AKI come from two experimental models: ischemia reperfusion injury (IRI) and cisplatin-induced AKI. These models highlight the roles of cytokines and leukocytes in mediating injury. This is sterile inflammation. The initiating trigger is followed by tissue stress and necrosis, initiating the production of pathogen-associated molecular pattern molecules (including hypoxia-inducible factor and high-mobility group box 1), inducing the upregulation of leukocyte adhesion molecules, chemokines, and TLR signaling (39). Leukocyte infiltration is rapid and significant, involving neutrophils, monocyte, macrophages, and a variety of T cells (including CD4+ Th1 cells, natural killer cells, NKT cells, $\gamma\delta$ T cells, and DCs) (40).

Macrophages, DCs, and MCs are potent producers of TNF-α, whereas MCs are the only leukocytes that store presynthesized TNF-α in granules. Blocking degranulation of MCs in cisplatin AKI with disodium cromoglycate prevented the increase of TNF-α in serum and protected from injury (41). Inhibition of TNF-α is also beneficial in endotoxin-, cisplatin-, and ischemia-induced AKI (42,43). IL-1 is responsible for enhancing neutrophil influx in IRI (44). NLRP3 inflammasome knockout (−/−) mice are protected against IRI but not in cisplatin-induced AKI (45). However, caspase-1−/− mice are protected from cisplatin-induced

Table 3. Clinical efficacy of biological inhibitors of cytokines

Cytokine Inhibition	Biologic	Effectiveness Proven	Ineffective
IL-1	Anakinra Canakinumab Rilonacept	Juvenile arthritis and cryopyrin-associated periodic syndromes	RA
IL-6	Tocilizumab	RA and juvenile arthritis	—
TNF-α	Adalimumab Certolizumab Etanercept Golimumab Infliximab	RA, juvenile arthritis, ankylosing spondylitis, IBD, and psoriasis	Multiple sclerosis and ANCA vasculitis
IL-12/IL-23	Ustekinumab Briakinumab	Ankylosing spondylitis, IBD, and psoriasis	—
IL-17	Secukinumab	Ankylosing spondylitis and psoriasis	RA and IBD

RA, rheumatoid arthritis; IBD, inflammatory bowel disease.

AKI (46), but IL-1$\beta-/-$ mice are not (47). The role of IL-6 in AKI is complex. Evidence in ischemic AKI is consistent with an injurious role for an endogenous IL-6 (48). However in an $HgCl_2$ model, it was shown that while IL-6–mediated inflammatory responses contributed to injury, IL-6 trans-signaling induced protective responses (49).

Cytokine-based immunomodulation can potentially be used as preventative or therapeutic in AKI. The therapeutic potential of administering anti-inflammatory cytokine IL-10 has been demonstrated to be effective in both ischemic and cisplatin-induced AKI (50). More recently, it has been appreciated that Tregs can protect from cisplatin (51) and ischemic AKI (52). Adoptive transfer of Tregs before cisplatin and before ischemia protected from the development of AKI. The number of Tregs required for protection in mice suggests the procedure is feasible in humans. Additionally, transferring Tregs to mice 24 hours after ischemic AKI was beneficial in promoting repair (53).

Cytokines released from kidneys with AKI can have significant effects on distal organs by circulatory spillover. Mortality in intensive care units is predicted by distal organ involvement in AKI. Recent studies suggest systemic proinflammatory effects are triggered in three waves by the immune release of host alarm signals (alarmins) from the AKI-damaged kidney (54). This begins with a uric acid surge, which induces a second wave of endothelial cell Weibel–Palade bodies released, which is then followed by a third wave of high-mobility group box 1 protein release. These events are potent triggers for GM-CSF, IFN-γ, CXCL8, G-CSF, IL-12, TNF-α, and IL-6 (55). Recently, renal DCs were implicated in inducing cytokine-mediated injury in ischemic AKI. Renal DCs can powerfully influence AKI by enhancing or attenuating injury. After injury, DCs initiate innate inflammatory responses presenting glycolipids and stimulating NKT cells, recruiting neutrophils and initiating the IL-17/IL-23 signaling pathway. DCs produce TNF-α, IL-6, IL-12, IL-23, IL-17, and IFN-γ to amplify injury and inflammation. However, adenosine A_{2A}Rc signaling can attenuate DC activation and protect from injury in ischemic AKI (56).

Autoimmune Crescentic Glomerulonephritis

Lupus Nephritis

SLE is a disease with evidence of genetic, epigenetic, and environmental contributions. There appears to be many different immune abnormalities associated with this disease, including significant abnormalities of cytokine circuits. It is also likely that there are multiple paths of autoimmunity depending in part on which component of immunomodulation is defective. Although it is likely that cytokines are therapeutic targets in this disease, the known presence of multiple immunoregulatory abnormalities suggests that SLE should not be considered as a single homogenous disease.

IFN-α. There is good evidence that IFN-α is an important inducer of antichromatin autoimmunity in patients developing SLE. Early in the disease, it has been demonstrated that immune complexes (containing DNA and/or RNA) are taken up by plasmacytoid DCs by FCγR-mediated internalization. Together with TLR7 and TLR9 stimulation, this induces IFN-α production, driving autoimmunity. Evidence supporting this comes from the high incidence of IFN-α–regulated genes in PBMCs of patients with SLE, the IFN-α signature (57). The levels of serum IFN-α and expression of IFN-α–regulated genes correlate with disease activity, autoantibodies, and complement levels (58). IFN-α signaling pathway polymorphisms have been shown in families with SLE (59). IFN-α is thought to be a promising therapeutic target for SLE, and several mAb inhibitors are in clinical trials. Sifalimumab is a humanized anti–IFN-α mAb. Its use in SLE was associated with reduction in SLE flares and activity (60). In a recent clinical trial, use of rontalizumab (another anti–IFN-α mAb) reduced the expression patterns of IFN-α–driven genes, improved disease severity, and improved flare rates in patients with SLE. However, patients with lupus nephritis were not included in this trial. AGS-009 is an IgG4 humanized mAb that induced significant attenuation of IFN-α signatures after a single dose (61). A novel approach has been to vaccinate patients with SLE with IFN-α–kinoid molecules to induce autoantibodies to IFN-α. All immunized patients returned the IFN-α signature to baseline (62).

TNF-α. Mouse models of lupus nephritis have shown both potentially protective and accentuating roles for TNF-α. New Zealand Black (NZB)/New Zealand White (NZW) and MRL/lpr mice with decreased synthetic capacity of TNF-α develop lupus nephritis, but in the kidney, intrarenal expression of TNF-α correlates with disease activity and inflammation (63). In NZB/NZW mice with IFN-α–induced nephritis, anti–TNF-α antibodies attenuate renal inflammation and injury despite maintained immune complex deposition (64).

The data on the role of TNF-α is conflicting; hence, the benefits of targeting TNF-α in human lupus nephritis is uncertain. However, there is the general view that there is sufficient data to advise against TNF-α inhibition. Several studies show that circulating levels of TNF-α and renal expression is increased (65); however, other studies show TNF-α production by PBMCs was lower in patients with lupus nephritis than controls (66). Additionally, TNF-α production was associated with reduced TNF-α bioactivity because of high serum levels of TNF receptors, which also correlated with increased disease activity (67). Finally, in patients with lupus nephritis, 10 weeks of infliximab treatment reduced proteinuria but increased anti-DNA antibodies. Longer treatment was associated with adverse effects (68). In patients with RA treated with anti–TNF-α mAbs, lupus syndromes developed, anti-DNAs were induced (69), and some patients developed GN (70).

IL-6. IL-6 is likely to act in concert with type 1 IFNs to induce B-cell autoimmunity in SLE. Its levels are elevated in lupus nephritis and correlate with disease activity (71). IL-6 has been demonstrated in glomerular immune complexes and proximal tubular epithelial cells in lupus nephritis (72). Intrinsic renal cells produce IL-6, and this can be enhanced by anti-DNA antibodies (73). In lupus-prone mice, IL-6 exacerbates GN while inhibiting IL-6 signaling attenuated GN and reducing autoimmunity, therefore enhancing survival (74). Most IL-6 inhibition trials have occurred in RA while data are emerging in SLE. Tocilizumab reduced acute phase reactants, anti–double-stranded DNA antibody, and SELENA-SLEDAI scores in patients with moderately active lupus nephritis (75).

A number of biologic interventions target molecules or immune cells upstream of cytokine production. The beneficial

effects are likely to result from their effects on cytokine mediation of target organ inflammation.

B-cell Activating Factor. B-cell activating factor is also known as B lymphocyte stimulator and is essential for B-cell maturation, survival, and Ig class switching. Its levels are elevated in SLE and correlate with disease activity and flares (76). Belimumab, a humanized anti-B lymphocyte stimulator mAb has been shown in two trials to demonstrate a modest but significant benefit in reducing disease activity. Patients with lupus nephritis were excluded, but in one trial, 15% of patients had evidence of nephritis. A *post hoc* analysis showed significant reduction in proteinuria. Trials in lupus nephritis are underway (77,78).

Abatacept. The data at hand do not provide evidence for CTLA4-Ig efficacy in the treatment of lupus nephritis. A phase IIb trial involving patients with lupus with polyarthritis and discoid lupus did not meet its primary or secondary end point, flare prevention, and the infection incidence was significantly higher in the abatacept arm (79). In another 12-month trial of abatacept or placebo, intravenous infusion plus steroid and mycophenolate mofetil were compared in patients with class III and class IV lupus nephritis. Complete response and renal improvement criteria were the same in all groups. Infection was not higher in the abatacept group (80).

Anti–TNF-Related Weak Inducer of Apoptosis. There is growing evidence for the anti–TNF-related weak inducer of apoptosis (Tweak)/factor inducible 14 pathway in enhancing injury in lupus nephritis. In lupus nephritis, Tweak and its receptor are upregulated in renal tubular cells, inducing proinflammatory cytokines, including IL-6, adhesion molecules, and chemokines (81). Immunoneutralization of Tweak decreases renal inflammation in murine models (82), and these mAb are being studied in lupus nephritis (ClinicalTrials.gov identifier: NCT0130890).

Laquinimod. Laquinimod is a small molecule that immunomodulates APCs to redirect Th subset differentiation with downregulation of IL-6, IL-12, IL-23, IL-17, and TNF-α and increased IL-10. In experimental murine lupus, laquinimod delayed the onset of lupus nephritis. When administered as a therapeutic, it attenuated disease severity by reducing IFN-γ and IL-17A production by splenocytes while enhancing IL-10 and Treg frequency (83). In a phase II study in active lupus nephritis, mycophenolate mofetil and high-dose steroid were administered with or without laquinimod. Laquinimod had an additive effect with renal function and proteinuria improvement. Adverse effects were not observed (84).

Despite many trials of therapeutics that attenuate cytokine action being performed in SLE, there is still much that needs to be understood about the role of cytokines in this disease. Unfortunately, the simple application of therapies successful in other immune inflammatory diseases (as in the case of anti–TNF-α immune neutralization) appears to be much more problematic in SLE. Finally, the diversity of patterns of disease and the involvement of different organs means lupus nephritis is unfortunately an exclusion in many trials, denying these patients the opportunity for potentially more effective treatments.

Experimental Antiglomerular Basement Membrane Glomerulonephritis. Experimental antiglomerular basement membrane (GBM) GN is the most widely studied animal model of human crescentic GN. There is considerable data showing that immune cytokines are critically involved in inducing nephritogenic autoimmunity and mediating glomerular injury in these models. Moreover, studies in this model provide proof of concept that the inhibition of selected cytokines can prevent and treat disease.

Numerous innate cytokines have been associated with pathogenesis of anti-GBM GN. A pathogenic role in anti-GBM GN has been demonstrated for each of the following innate cytokines: GM-CSF, G-CSF, IL-1β, TNF-α, and CXCL8, by studies in cytokine gene–deleted mice. All of these innate cytokines recruit inflammatory cells to the kidney and direct the subsequent development of anti-GBM GN. Gene deletion of the key Th1 cytokines (IL-12 and IFN-γ) resulted in attenuated crescentic GN, and gene deletion of the key Th1 transcription factor (T-bet) is protective (85,86). After the discovery of the CD4+ Th17 subset, studies using mice deficient in p19, p35, and p40 (components of the key Th1 and Th17 cytokines, IL-12 and IL-23, respectively) were used to analyze the relative contributions of each Th subset in anti-GBM GN. Paust *et al.* demonstrated that Th17 cells contributes to anti-GBM GN with the use of IL-23p19−/− and IL-17A−/− mice (87), whereas Odobasic *et al.* examined the reciprocal relationship between Th1 and Th17 and demonstrated that early nephritogenic responses were mediated by Th17 cells, but late disease is Th1 dependent. They also demonstrated that each Th subset counterregulated the other (88). Furthermore, Steinmetz *et al.* used mice with gene deletion of RORγ-t, the key Th17 transcription factor, to confirm the participation of the CD4+ Th17 subset (89). Direct comparison has been made between the effects of transferring Th17 and Th1 polarized ovalbumin (OVA) specific CD4+ TCR transgenic cells into naïve mice with OVA planted on the GBM using a non-nephritogenic anti-GBM/OVA conjugated antibody. Transfer of both CD4+ T-cell clones induced GN. However, transfer of Th1-polarized cells induced a monocyte and macrophage predominant infiltrate lesion, whereas Th17-polarized cells induced less injury with a neutrophil predominant infiltrate (90). To assess key Th2 cytokines, anti-GBM GN was induced in IL-4−/− and IL-10−/− mice. Both groups had augmented Th1 responses and increased glomerular injury, whereas infusion of IL-10 attenuated disease. Tregs are not only important in maintaining self-tolerance but are also necessary in controlling overt inflammatory responses. Transfer of Tregs (CD4+ CD25+) before and after the induction of experimental anti-GBM GN suppressed the development of GN by reducing Th1 responses (91). Interestingly, Eller *et al.* demonstrated that Treg-derived IL-9 is essential for the recruitment of MCs, and both are required to attenuate anti-GBM GN (92).

Cytokine Production by Resident Renal Cells. Resident cells within the kidney, including tubular and glomerular cells, interact with infiltrating leukocytes resulting in their synthesis of injurious TNF-α in response to leukocyte-produced IL-1 (73). The relative roles of leukocyte and resident cytokine production have been studied in anti-GBM GN in mice using cytokine chimeric mice where the cytokine gene has been deleted from either bone marrow–derived leukocytes or from resident renal cells (86). Nonchimeric TNF-α−/− mice are significantly protected from the development of GN. When the TNF-α gene is knocked out from resident cells, similar attenuation was observed,

whereas knockout of the TNF-α gene in bone marrow only caused mild protection, suggesting that TNF-α produced by resident cells is the major source of injurious TNF-α in this disease. These studies also produced evidence of complex cytokine interactions between resident cells and infiltrating leukocytes. Studies with IL-1β−/− and IL-1R1−/− mice showed that IL-1β mainly derived from leukocytes actives IL-1R1 on resident cells, inducing TNF-α production that causes significant glomerular injury (86) (Figure 2).

Autoimmune Anti-GBM Glomerulonephritis. In autoimmune anti-GBM GN, the target autoantigen is the non-collagenase domain of the alpha 3 chain of type IV collagen. Immunization with this antigen induces autoimmunity shown by the production of circulating anti-GBM antibodies and the development of crescentic GN. IFN-γ−/− mice developed worse disease. The relative contributions of Th1 and Th17 to disease development were assessed using p35−/− (deficient in Th1 subset) and p19−/− (deficient in Th17 subset) mice. The p19−/− mice were protected from GN, but the p35−/− mice were unaffected compared with controls, confirming a pathogenic role for Th17 but not for Th1 in this disease.

ANCA-Associated Crescentic Glomerulonephritis. The most common cause of crescentic GN is ANCA-associated vasculitis (AAV). Evidence suggests cytokines directing the underlying nephritogenic autoimmunity are likely to be therapeutic targets that could be neutralized with available biologic agents.

PBMCs from patients with ANCA-associated GN have CD4+ T cells that proliferate when stimulated with the target autoantigens, proteinase 3 or myeloperoxidase (MPO) (93). In treatment of refractory disease with T cell–specific targeted therapies, antithymocyte globulin was beneficial and capable of inducing remission (94). Furthermore, cytokine profiling of biopsied nasal mucosal tissue, bronchoalveolar lavage, and PBMCs from patients with granulomatous polyangiitis demonstrated increased expression of IFN-γ, denoting a Th1 cytokine pattern (95). Nogueira *et al.* found that in the serum of acute or convalescent AAV patients, IL-17A and IL-23 levels were increased, and this correlated with disease severity and ANCA titer (96). IL-6 was also elevated in active disease. Chavele *et al.* reported that in patients with MPO-AAV, MPO-stimulated recall responses showed elevated IFN-γ (97). Collectively, these data provide evidence in support of both Th1 and Th17 involvement in AAV. Furthermore, IL-17–producing cells were found in renal biopsies from patients with acute vasculitis, and most of the IL-17–positive cells present were innate leukocytes (neutrophils and MCs) (98).

In experimental MPO-ANCA GN, IL-17A−/− mice were protected from the development of anti-MPO autoimmunity and glomerular injury (99). Immunoneutralization of TNF-α caused significant reduction in lung hemorrhage and renal injury in Wistar–Kyoto rats with induced anti-MPO AAV (100).

The only cytokine-based clinical trials in AAV have been of anti–TNF-α. Treatment with etanercept (anti–TNF-α mAb) was not effective and was associated with a high rate of treatment-related adverse side effects (101). A smaller clinical trial using adalimumab with prednisolone

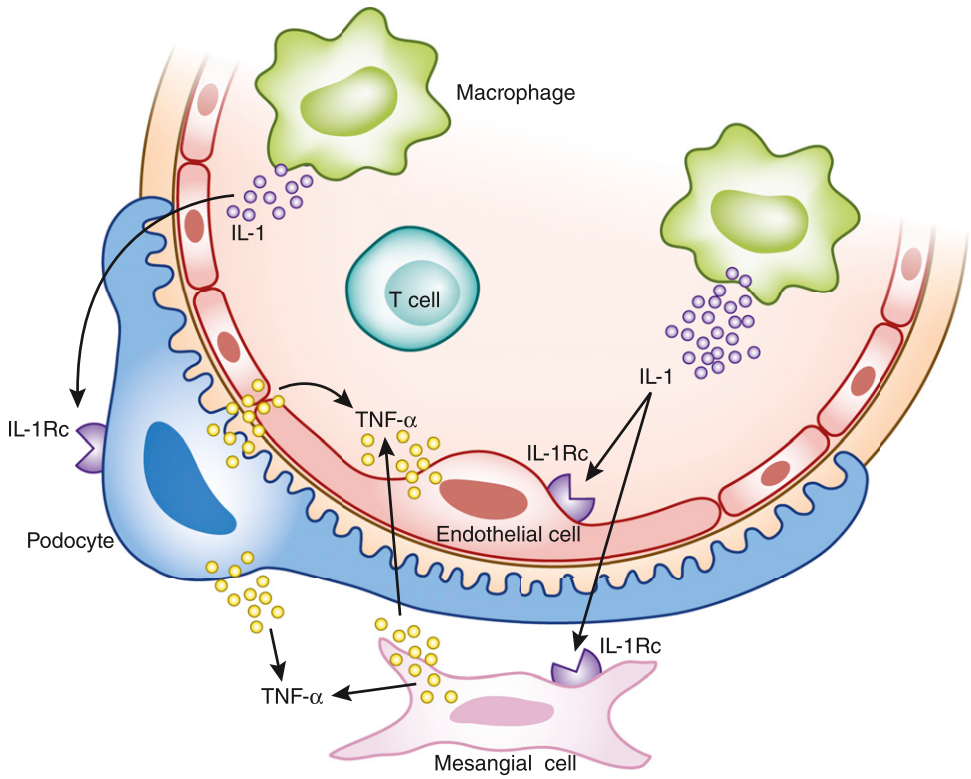

Figure 2. | Innate macrophages produce IL-1 which binds to IL-1Rc on intrinsic renal cells (endothelial cells, podocytes, and mesangial cells) to produce injurious TNF-α that amplifies T effector cell responses resulting in crescent formation and glomerular injury. Rc, receptor.

plus cyclophosphamide showed similar efficacy to using these drugs but afforded with less steroid exposure (102).

Opportunities for Introducing Biologic Therapies to Treat Renal Diseases

The evidence outlined here suggests that there are important renal diseases that may benefit from the application of well targeted biologic therapies on the basis of cytokine manipulation.

Innate cytokines are prominent participants in many forms of AKI. Moreover, in some clinical settings, such as renal transplantation, high-risk surgery (*e.g.,* elective coronary grafting), and for optimal use of effective chemotherapeutics (nephrotoxic dose-limited like cisplatin), the opportunity exists to preemptively block likely injurious cytokine pathways.There is a need for more selective, less toxic therapies to treat autoimmune inflammatory forms of proliferative/crescentic GN, including lupus nephritis, anti-GBM, and ANCA-associated disease. These represent the more serious and inflammatory categories of theses autoimmune diseases. They share features of other diseases, such as RA and IBD, where new biologics are now part of the therapeutic pharmacopeia.

The initial attempt to introduce anti–TNF-α mAbs in AAV was disappointing. It should remind us that translation of therapies from one disease to another is not simple or without risk, but without well managed risk, there will be no progress. Perhaps we should apply the principles behind the introduction of anti–TNF-α to RA, use tissue samples from patients with active disease to assess the dominant cytokines in active untreated disease, assess the effects of these cytokines on relevant renal tissues *in vitro*, and use relevant animal models to provide proof of concept for the specificity, efficacy, and minimal toxicity in preclinical trials. Clinical trials with the greatest likelihood of success are those that target dominant cytokines in renal diseases using immunoneutralising mAbs where minimal toxicity and clinical effectiveness has already been shown in other chronic autoimmune and or autoinflammatory disease. Using these criteria we could now be planning clinical trials for targeting the major innate cytokine as prophylaxis and treatment and considering neutralizing mAbs to IL-17 and IL-23 in ANCA vasculitis.

Disclosures

None.

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B Cells, Antibodies, and More

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Abstract

B cells play a central role in the immunopathogenesis of glomerulonephritides and transplant rejection. B cells secrete antibodies that contribute to tissue injury *via* multiple mechanisms. In addition, B cells contribute to disease pathogenesis in autoimmunity and alloimmunity by presenting antigens as well as providing costimulation and cytokines to T cells. B cells also play an immunomodulatory role in regulating the immune response by secreting cytokines that inhibit disease onset and/or progression. B cell–targeted approaches for treating immune diseases of the kidney and other organs have gained significant momentum. However, much remains to be understood about B-cell biology in order to determine the timing, duration, and context of optimal therapeutic response to B cell–targeted approaches. In this review, we discuss the multifaceted roles of B cells as enhancers and regulators of immunity with relevance to kidney disease and transplantation.

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Introduction

Historically, immune responses have been classified as cellular or humoral. Cellular responses are mediated by T lymphocytes, which recognize and attack their targets directly or indirectly by enlisting the help of other immune cells, while humoral responses are characterized by the production of antibodies by B lymphocytes and their progeny, plasma cells. These antibodies permeate extracellular spaces, where they protect against infection and also contribute to tissue injury in autoimmunity and transplantation. B cells have therefore traditionally been associated with humoral immunity, but we now know that they are equally critical to cellular immunity. B cells participate in T-cell activation *via* antigen presentation, costimulation and cytokine production; affect antimicrobial defenses and tissue inflammation; and, importantly, serve as regulatory cells that modulate both cellular and humoral responses. Here, we review the classic humoral and the more recently described cellular functions of B cells, with particular emphasis on their roles in the pathogenesis of GN, transplant rejection, and AKI.

Primer in B-Lymphocyte Biology

B-Lymphocyte Lineage Subsets

Three principal classes of B lymphocytes exist in mice and humans, classified on the basis of their ontogeny and anatomic localization: B1 and B2 B lymphocytes, consisting of the marginal zone (MZ) and follicular (FO) B cells (Figure 1). B1 lymphocytes arise from B1 progenitors in fetal liver and persist as a self-renewing population beyond the neonatal period, with little input from the bone marrow (BM) in adulthood, while B2 lymphocytes develop from transitional 2 (T2) B cells that originate from BM precursors with continued output throughout life (1–4). In mice, B1 B cells predominantly reside in the peritoneal and pleural cavities and produce IgM antibodies directed against so-called thymus- or T-independent antigens, usually carbohydrate or phospholipid antigens present on commensal bacteria. They are called T independent because they do not require T-cell help to elicit antibody production. Such antibodies are polyreactive or polyspecific in that they can bind to both self-antigens and microbial antigens.

A prototypical example of antibodies secreted by B1 B cells are those directed against ABO blood groups, which arise naturally during the first few months of life because of structural similarities between the ABO system and bacterial carbohydrate antigens recognized by B1 B cells (5,6). Natural IgM antibodies secreted by B1 B cells play an important role in maintaining tissue homeostasis because of their ability to bind altered self-antigens, such as those expressed by apoptotic cells in ischemia-induced tissue injury and oxidized LDLs in atherosclerosis (7). In addition to IgM, B1 B cells also produce polyreactive IgA antibodies that contribute to mucosal immunity along with IgA secreted by FO B cells (8). Although the existence of B1 B cells as a distinct lineage in humans has been controversial, B cells expressing CD5 that are the source of poorly glycosylated IgA1 and thought to be B1 B cells are increased in patients with IgA nephropathy and contribute to disease pathogenesis (9–11).

MZ B cells develop from transitional B cells after induction of neurogenic locus notch homolog protein 2 (NOTCH2) and engagement of its ligand delta-like 1 on endothelial cells, with subsequent retention within the marginal sinus of the spleen mediated by sphingosine-1-phosphate, integrins lymphocyte function–associated antigen 1, and very late antigen 4 ($\alpha4\beta1$-integrin, CD49d/CD29), and cannabinoid receptor 2 (4). MZ B cells express polyreactive B-cell receptor (BCRs), complement receptors (CD21 and CD35), and MHC class 1–like molecule CD1d; they

produce polyreactive IgM antibodies that facilitate clearance of blood-borne microorganisms and apoptotic cells (4). Similar to B1 B cells, MZ B cells recognize T-independent carbohydrate and phospholipid antigens, a classic example being the recognition of pneumococcal capsular polysaccharides; thus, the susceptibility of splenectomized individuals to systemic pneumococcal infection (12). Both B1 and MZ B cells constitutively express Toll-like receptors (TLRs) and can readily respond to pathogen-associated or endogenous TLR ligands, with or without antigen recognition *via* their BCR. Thus, B1 and MZ B cells respond like innate cells in mediating rapid IgM antibody responses (approximately 1–3 days) that bridge the temporal gap in immunity against infections until the emergence of FO B cell–derived IgG antibodies (about 7 days). Unlike B1 B cells, MZ B cells also participate in responses to T-dependent protein antigens by generating high-affinity isotype switched antibodies and transporting complement-bound opsonins onto FO dendritic cells (DCs) in splenic follicles aiding germinal center (GC) reactions (13). MZ B cells thus represent a versatile population in their ability to rapidly generate antibodies *via* not only T-independent but also T-dependent pathways that were previously attributed solely to FO B cells. Abnormal increases in B1 and MZ B cells are described in murine models as well as in patients with autoimmune diseases, including lupus (3,4,14).

Finally, FO B cells, which reside in spleen and lymph nodes, are the conventional B lymphocytes of the adaptive immune system and are the most numerous of all B cell lineages. FO B cells arise from transitional B cells in the spleen through a pathway dependent on Bruton tyrosine kinase induced by BCR-mediated signals (2). Although FO B cells participate in T-independent IgM responses, they are primarily responsible for the generation of long-lasting, high-affinity IgG antibodies with the help of T lymphocytes, critical for classic humoral immunity mediating protection after infection or vaccination. As will be discussed later, FO B cells specific to self-antigens or transplantation antigens also play a key role in the pathogenesis of autoimmune kidney disease and transplant rejection.

Antibody Types

Because a principal function of B lymphocytes is antibody production, it is important at this point to summarize the salient features of these defense molecules and describe their different isotypes or classes. Antibodies, also known as immunoglobulins, are glycosylated protein molecules present on the surface of B cells (surface immunoglobulins) serving as antigen receptors (BCR), or are secreted into the extracellular space where they can bind and neutralize their target antigens (15). A single antibody molecule consists of four protein chains: two "heavy" and two "light," linked to each other by disulfide bonds (Figure 2). The N-terminus regions of the heavy and light chains, which collectively make up the antigen-binding site, are where the variability between one antibody molecule and another resides, hence determining specificity.

Five isotypes, or classes, of antibodies (IgM, IgD, IgG, IgA, and IgE) exist, and they are distinguished according to the C-terminus regions of the heavy chains, which are constant and therefore do not participate in antigen binding. Instead, these regions (designated Fc) are important for the effector

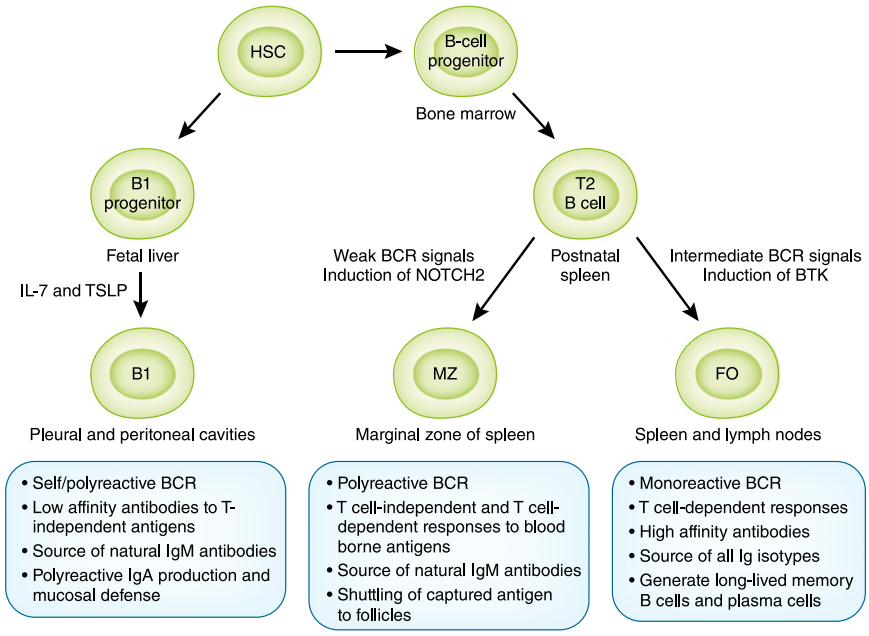

Figure 1. | **B-cell lineage subsets and functions**. B lymphocytes of all lineages arise from progenitors derived from hematopoietic stem cells (HSCs). Most B1 B lymphocytes develop from B1 progenitors in the fetal liver with little input from bone marrow beyond the perinatal period. B2 B lymphocytes develop from transitional 2 (T2) B cells derived from B-cell progenitors in the bone marrow, with subsequent differentiation into marginal zone (MZ) and follicular (FO) lineages occurring in the spleen. Stronger B-cell receptor (BCR) signals induce Bruton tyrosine kinase (BTK) and support maturation to FO B cells, while weaker BCR signals allow expression of neurogenic locus notch homolog protein 2 (NOTCH2) giving rise to MZ B cells. B lymphocytes of each lineage have distinct and overlapping functions in recognizing antigens *via* T-independent and T-dependent pathways, production of rapid IgM, and long-lasting IgG antibody responses essential for host defense.

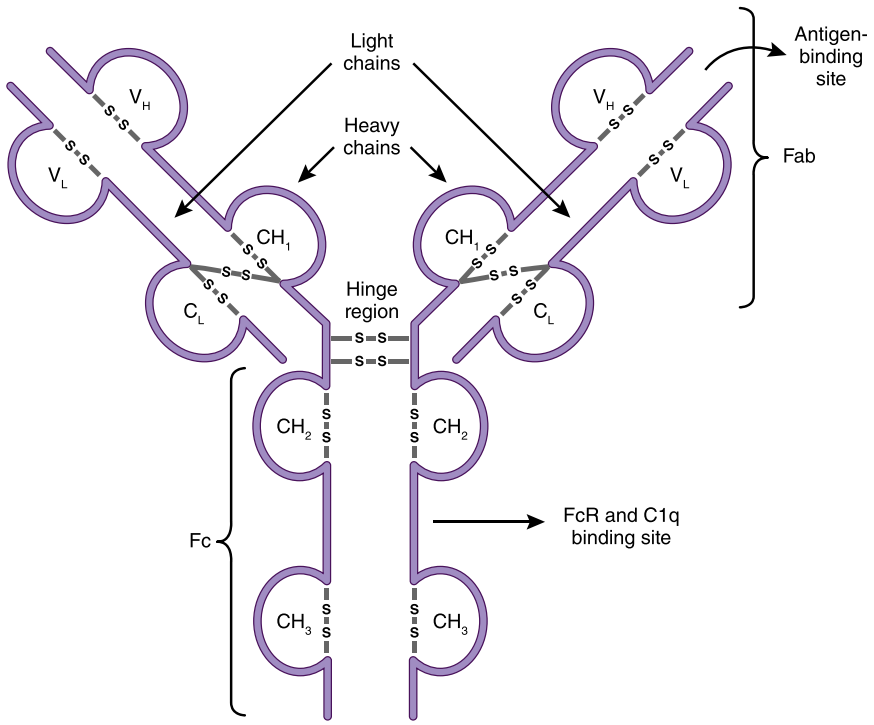

Figure 2. | **Antibody structure**. Antibodies (immunoglobulins) are composed of two heavy chains (V_H and C_H) and two light chains (V_L and C_L). The antigen-binding fragment, Fab, is composed of one variable domain from each heavy and light chain (V_H and V_L). The variable domains contain the complementarity determining regions (CDRs) with the most sequence variations and determine antibody specificity. The constant domains CH2 and CH3 of the heavy chain make up the crystallizable fragment, Fc, which mediates effector functions through binding to Fc receptors (FcRs) on cells and to complement (C1q).

functions of antibodies, the means by which antibodies eliminate pathogens or alternatively cause tissue injury. In addition, there are four subclasses or isotypes of IgG antibodies (IgG1, IgG2, IgG3, and IgG4). Antibodies exert effector functions in three principal ways: They neutralize their targets (*e.g.*, they bind to a virus and prevent it from entering a cell), they activate macrophages and other immune cells by binding to Fc receptors (FcRs) that recognize the constant regions of specific antibody classes, or they activate the classic pathway of the complement system by binding to C1q (Table 1). Which effector mechanism dominates is determined by the heavy-chain isotype and binding affinities of activating and inhibitory FcR on immune cells. For example, IgM and IgG3 are excellent complement activators, while IgG1 and IgE bind FcR to activate macrophages and mast cells, respectively (15).

Table 1. Immunoglobulin isotypes and functions

Characteristic	Immunoglobulin Isotype						
	IgM	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Heavy chain	μ	γ_1	γ_2	γ_3	γ_4	α	ε
Molecular mass, kDa	970	146	146	165	146	160	188
Serum level (mean adult), mg/ml	1.5	9	3	1	0.5	2.1	5×10^{-5}
Half-life in serum, days	10	21	20	7	21	6	2
Polysaccharide antigens	++	+	+++	+/−	+/−	++	++
Protein antigens	+	++	+/−	++	++	+	+
Placental transfer	–	+++	+	++	−/+	–	–
Neutralization	+	++	++	++	++	++	–
Classic pathway of complement activation	++++	++	+	+++	–	–	–
Sensitization for killing by natural killer cells	–	–	++	–	++	–	–
Binding to macrophage and phagocyte Fc receptors	–	+	–	+	−/+	+	+
Binding to mast cells and basophils	–	+	–	+	–	–	+++

+ denotes relative presence and – denotes relative absence of response to type of antigen or specified characteristic.

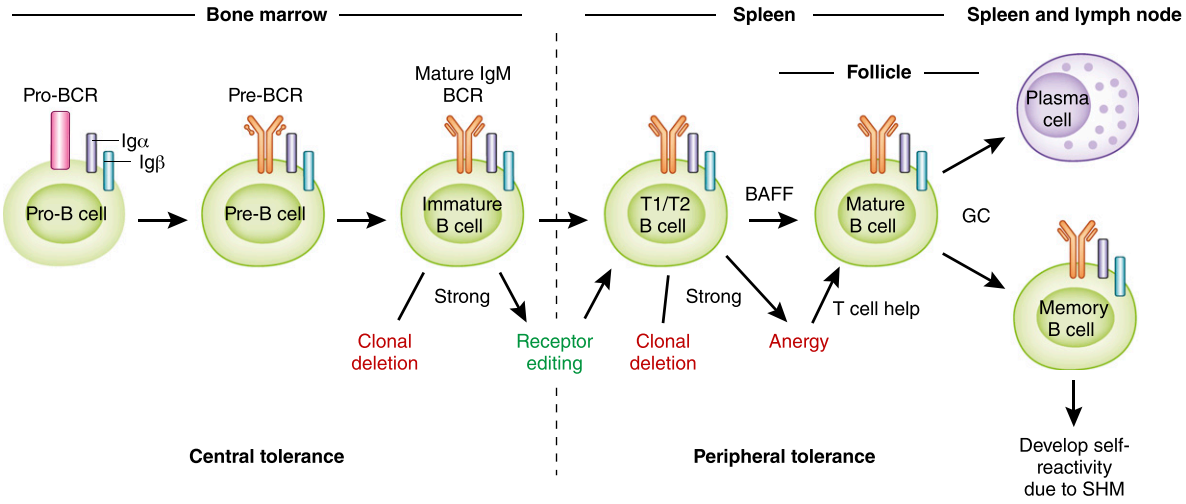

Figure 3. | **B-cell development and mechanisms of self-tolerance**. B-cell development begins in the bone marrow and is completed in peripheral lymphoid tissues, such as the spleen. Development in the bone marrow progresses sequentially through pro-B, pre-B, and immature B cell stages and expression of surface IgM, mature B-cell receptor (BCR). Immature B cells with strong reactivity to self-antigen undergo clonal deletion or rearrange their immunoglobulin gene segments; this is called receptor editing, which eliminates self-reactivity and allows entry to the transitional B-cell pool. Transitional B cells depend on B cell–activating factor (BAFF) for survival and differentiate into mature B cells in the spleen. Those transitional 1 and 2 (T1/T2) B cells with strong self-reactivity undergo clonal deletion or remain outside splenic follicles as hyporesponsive anergic B cells that can be rescued upon receiving T cell help to enter the mature B-cell pool. Mature B cells that are activated by foreign antigen and enter germinal center (GC) reactions give rise to isotype-switched memory B cells and plasma cells. During the process of somatic hypermutation (SHM), a few memory B cells acquire self-reactivity due to random immunoglobulin gene rearrangements and persist as IgG+ self-reactive clones in the periphery.

Pathogenic antibodies in patients with autoimmunity, such as lupus and transplant rejection, are usually IgG, with the isotype influenced by the nature of the antigen (*e.g.*, polysaccharide antigens incite IgG2, whereas protein antigens induce IgG1; Table 1) and concomitant cytokine milieu of the immune response (*e.g.*, IL-4 and IL-21 induce IgG1 and IgG3) (16). Among IgG isotypes, IgG1 and IgG3 bind FcγR most efficiently and also activate complement, contributing to their associated proinflammatory effects. IgG1 is the predominant isotype, constituting 60%–75% of serum IgG and has a longer half-life (3 weeks), providing the basis for the commonly used dosing regimens (every 3–6 weeks) of intravenous immunoglobulin (IVIG) when used as replacement in immunoglobulin deficiencies or treatment of autoimmune diseases. Efficient binding of IgG to its FcγR is influenced by post-translational modifications of the sugar moieties attached to the CH2 domain of the Fc fragment, affecting structural stability and function (17).

Differences in glycosylation of antibody molecules due to altered expression of glycosyltransferases are observed in various disease states and contribute to pathogenesis (18). For example, poorly galactosylated IgA1 aggregates form immune complexes with IgG that trigger a cascade of proinflammatory events upon binding to mesangial cells in IgA nephropathy (19); nonfucosylated IgG-Fc, which increases binding to FcγRIIIa and antibody-dependent cellular cytotoxicity, is observed in patients with antiplatelet alloantibodies and controllers of HIV infection (20,21); degalactosylated IgG is found in several autoimmune diseases, suggesting its pathogenicity; and increased terminal sialic acid residues linked to IgG Fc fragment confer potent anti-inflammatory properties of IVIG by binding to DC-specific intercellular adhesion molecule 3–grabbing nonintegrin expressed on macrophage and DC subpopulations, and causing upregulation of the inhibitory FcγRIIb (22–24). In addition to changes in glycosylation, binding affinity to FcR is also influenced by polymorphisms in activating (*e.g.*, FcγRIIIa) and/or inhibitory (*e.g.*, FcγRIIb) FcRs and contributes to pathogenesis in autoimmune diseases such as lupus (25,26).

B-Lymphocyte Development and Mechanisms of Self-Tolerance

B lymphocytes primarily originate in the BM, except for B1 B cells, which arise from fetal liver as previously discussed (1,3). B lymphocytes develop from common lymphoid progenitors of hematopoietic stem cells, which also give rise to T lymphocytes and natural killer cells with commitment to B-cell lineage being determined by the expression of paired box protein 5 (*Pax5*) (27). B-cell development progresses through sequential maturation steps within the BM before release of immature B cells into the circulation and subsequent completion of differentiation into mature B cells within the spleen. Developing B cells progress through rearrangement of immunoglobulin heavy- and light-chain gene segments (variable V, diversity D, joining J) from pro-B to pre-B to immature B cells, culminating in the expression of IgM mature BCR on the cell surface that can bind antigens (Figure 3) (28). The maturation steps depend on close interactions between developing B cells and BM stromal cells, which provide critical adhesive integrins, growth factors, chemokines, and cytokines (*e.g.*, Fms-like tyrosine kinase 3, thrombopoietin, C-X-C motif chemokine ligand [CXCL] 12, and IL-7) (27). Immature B cells exiting the BM

home to the spleen, where they differentiate into transitional 1 and 2 B cells, which mature into MZ or FO B cells guided by BCR signals, B cell–activating factor (BAFF), and expression of transcription factors, NOTCH2 and BTK (2,4,28). MZ B cells are retained in the spleen while FO B cells recirculate, populating various secondary lymphoid tissues (*e.g.*, lymph nodes, tonsils, and gut-associated lymphoid tissues, such as Peyer patches).

The random rearrangement process of immunoglobulin genes during B-cell development ensures the generation of a vast repertoire of BCRs capable of recognizing a huge diversity of antigens. This results in inherent generation of B cells that also recognize various self-antigens. In fact, 75% of immature B cells in humans are estimated to be self-reactive (29). These self-reactive or autoreactive B cells must be eliminated during development to avoid autoimmunity, while still preserving a diverse BCR repertoire in the mature B-cell pool essential for host defense. Developing B cells transit through several selection processes in the BM and spleen that serve as checkpoints in purging autoreactive clones and establishing self-tolerance (28). BCR recognition of self-antigen within the BM and the threshold of generated signals determine selection of immature B cells to move forward to the transitional B-cell stage: positive selection of clones with low-level (also referred to as "tonic") BCR signals; clones with no BCR signals fail to survive; and clones with strong signals are targeted for apoptosis (clonal deletion, also termed negative selection), unless they rearrange their light-chain immunoglobulin gene segments (termed receptor editing), and re-express a

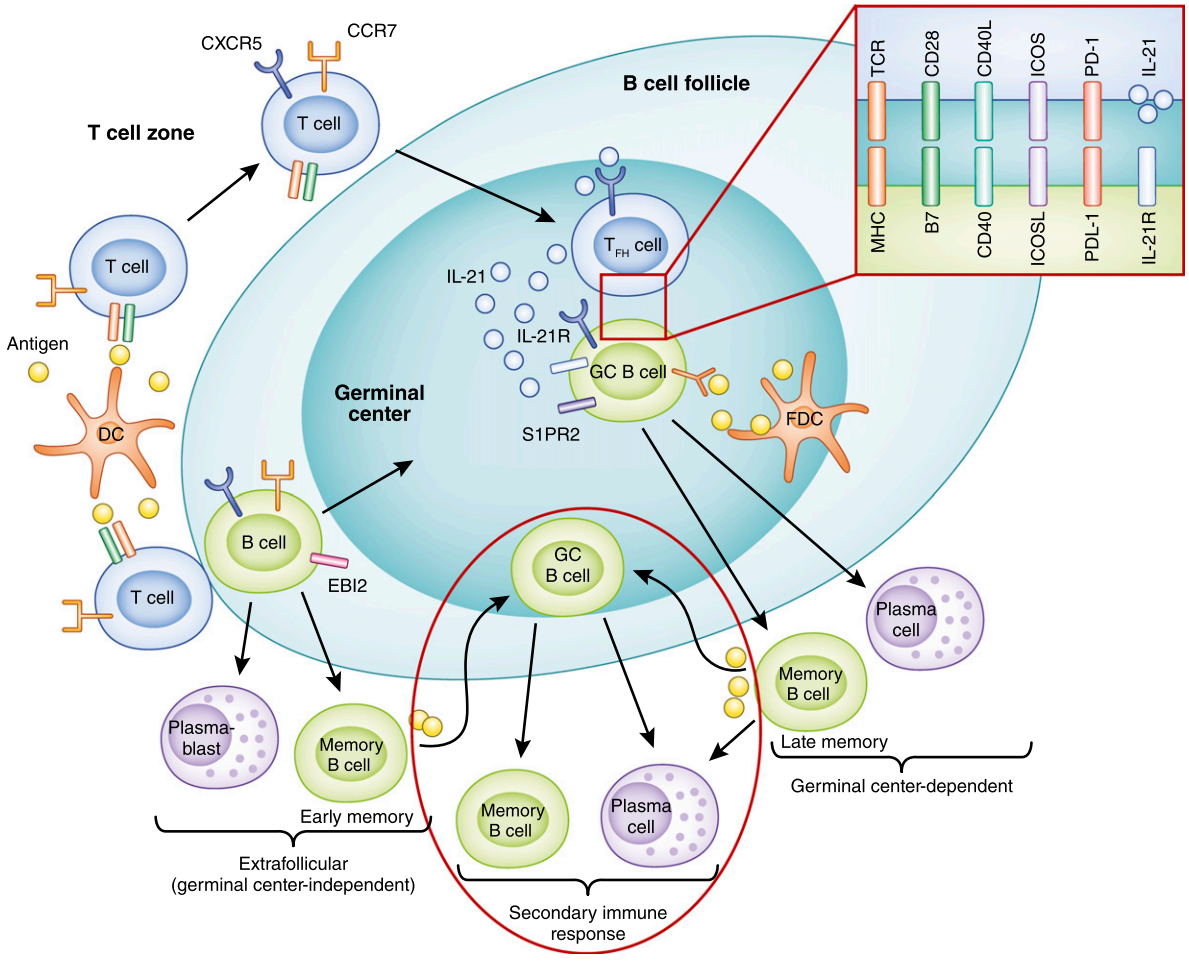

Figure 4. | **B-cell activation and differentiation into memory B cells and plasma cells**. B cells that have encountered antigen migrate to the T–B border by upregulating C-C chemokine receptor 7 (CCR7) and Epstein-Barr virus–induced receptor 2 (EBI2), where they first encounter cognate T cells that mature into T follicular helper cells (Tfhs). B cells can differentiate into extrafollicular plasma blasts or memory B cells independent of germinal centers (GCs). B cells that express *B-cell lymphoma 6* (*Bcl6*) return to the follicles, where they are retained *via* sphingosine-1-phosphate receptor 2 (S1PR2) expression to form GCs with Tfhs. Within GCs, B cell–Tfh interactions *via* MHC 2–T-cell receptor, B7–CD28, CD40–CD40L, inducible costimulator ligand (ICOSL)–inducible costimulator (ICOS), programmed cell death protein ligand 1 (PDL1)–programmed cell death protein 1 (PD1), and IL-21 receptor (IL-21R)–IL-21 facilitate somatic hypermutation and immunoglobulin isotype class-switch recombination (CSR) that generate high-affinity GC-dependent memory B cells and long-lived plasma cells. Following antigen re-exposure, extrafollicular memory B cells now enter GCs to generate isotype-switched and high-affinity secondary memory B cells and plasma cells, while GC-dependent memory B cells can rapidly differentiate into secondary plasma cells or re-enter GC to produce secondary memory B cells and plasma cells. DC, dendritic cell.

BCR that now meets the threshold for positive selection into the transitional B-cell pool (Figure 3).

B-cell repertoire modification of immature B cells that occurs within the BM by clonal deletion and receptor editing is termed central tolerance, and the latter mechanism contributes to elimination of a majority of self-reactive clones (20%–50%). Additional selection mechanisms occurring within the spleen remove the remaining autoreactive clones that recognize peripheral self-antigens: Transitional B cells with strong BCR signals undergo clonal deletion or attain a state of hyporesponsiveness, termed anergy, with shortened survival (1–5 days) (Figure 3) (30). However, these peripheral tolerance mechanisms can be circumvented by elevated levels of BAFF and T-cell help of anergic B cells, enabling autoreactive clones to enter the mature B-cell pool (31). Self-reactive B lymphocytes that escape clonal deletion, receptor editing, or anergy are eliminated by CD4+ T cells *via* Fas receptor–Fas ligand and CD40–CD40L interactions in addition to being held in check by CD4+ T cells and B cells with regulatory properties (Tregs and Bregs, respectively) (32,33). Failure of one or more of the self-tolerance checkpoints described earlier is central to development of autoimmune diseases, such as lupus, that also affect the kidneys (31). For example, in patients with systemic lupus erythematosus (SLE), defects in BCR signaling due to mutations in *BTK* or protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*) gene polymorphism disrupt central tolerance, and elevated serum BAFF levels and Fas receptor–Fas ligand polymorphisms contribute to observed defects in peripheral tolerance (31,34). Despite the absence of autoreactive clones entering the naive B cell pool, following foreign antigen-mediated activation and GC reaction, some IgG+ memory B cells acquire self-reactivity as a consequence of somatic hypermutation that also contribute to autoantibodies in SLE (31).

B-Lymphocyte Activation and Differentiation

A hallmark of humoral immunity is the generation of long-lived memory B cells and plasma cells that produce high-affinity, isotype-switched antibodies essential for host defense. B-cell activation and differentiation into extrafollicular or GC-driven memory B cells, plasma blasts, or plasma cells are guided by integration of (*1*) nature of antigen, such as polysaccharide, glycolipid, or protein; (*2*) associated TLR signals; and (*3*) cytokine and costimulatory helper signals (35). Polysaccharide and glycolipid antigens are poor activators of T cells, and in general, B1 and MZ B cells responding to these antigens are activated independent of conventional T-cell help. However, unlike FO B cells, B1 and MZ B cells express TLR in their nascent state, which allows them to integrate signals from TLR ligands (such as LPS, Cytosine-phosphate-Guanine DNA, and double-stranded RNA) derived from pathogens or damaged cells, along with antigen recognition, to differentiate rapidly into IgM or isotype-switched short-lived plasma blasts and memory B cells in extrafollicular areas without entering the GC (36). MZ B cells also interact with other helper cells, such as natural killer T cells, neutrophils, and

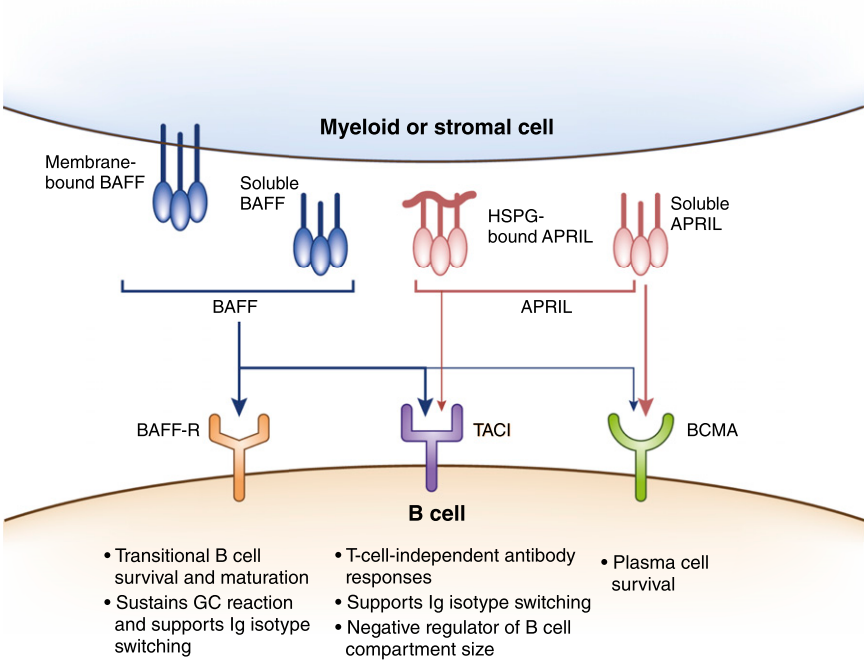

Figure 5. | B cell–activating factor (BAFF), a proliferation-inducing ligand (APRIL), and their receptors. BAFF and APRIL are transmembrane proteins of the TNF family that can be proteolytically cleaved to produce soluble forms. They are produced by myeloid cells, such as dendritic cells (DCs), neutrophils, monocytes, macrophages, and stromal cells. BAFF binds strongly to receptors, B cell–activating factor-receptor (BAFF-R) and transmembrane activator and cyclophilin ligand interactor (TACI), and weakly to B-cell maturation antigen (BCMA), whereas APRIL binds strongly to BCMA and moderately to TACI. APRIL can also exist bound to heparin sulfate proteoglycan (HSPG) in extracellular matrix and interacts with TACI in this form. BAFF promotes survival and maturation of transitional B cells into mature B cells, supports B cell proliferation, class-switch recombination (CSR), and plasma cell survival. APRIL is critical for T-independent responses and supports CSR and survival of plasma cells.

DCs, that provide cytokines (BAFF, a proliferation-inducing ligand [APRIL], IL-21, IL-6, and IL-10) and costimulatory signals (CD40L) within the extrafollicular areas, facilitating limited somatic hypermutation and antibody diversification (36). Thus, B1 and MZ B cells generate predominantly low-affinity IgM or isotype-switched IgG antibodies in extrafollicular areas independent of conventional T-cell help (4).

For protein antigens that are recognized primarily by FO B cells, activation is initiated upon antigen recognition by the BCR and critical helper signals derived from antigen-specific CD4 T cells (Figure 4). Upon binding antigen, the BCR sets two key processes in motion. First, it signals to the cell's interior to trigger essential gene expression programs. Second, it internalizes the antigen and delivers it to endosomal compartments, where it is degraded into peptides that are then bound to MHC-2 molecules and recycled to the surface of the B lymphocyte. These peptide–MHC-2 complexes are what antigen-specific CD4 T cells recognize to establish intimate contacts with B cells and provide them with the help needed for their proliferation and differentiation. Because the CD4 T cell providing help is activated by the same antigen as the B cell, the contact and interaction between these T and B cells is referred to as "cognate" or "linked." T–B interactions required for B lymphocyte activation are orchestrated not only in time but also in space (37). They take place within secondary lymphoid tissues guided by the expression of chemokine receptors and corresponding ligands (38). Naive B cells, for example, express C-X-C motif chemokine receptor 5 and are retained in clearly delineated areas, called primary lymphoid follicles or B-cell zones, in lymph nodes by CXCL13 from FO DCs (38). After antigen recognition, B cells upregulate C-C chemokine receptor 7 and Epstein-Barr virus–induced receptor to migrate to the

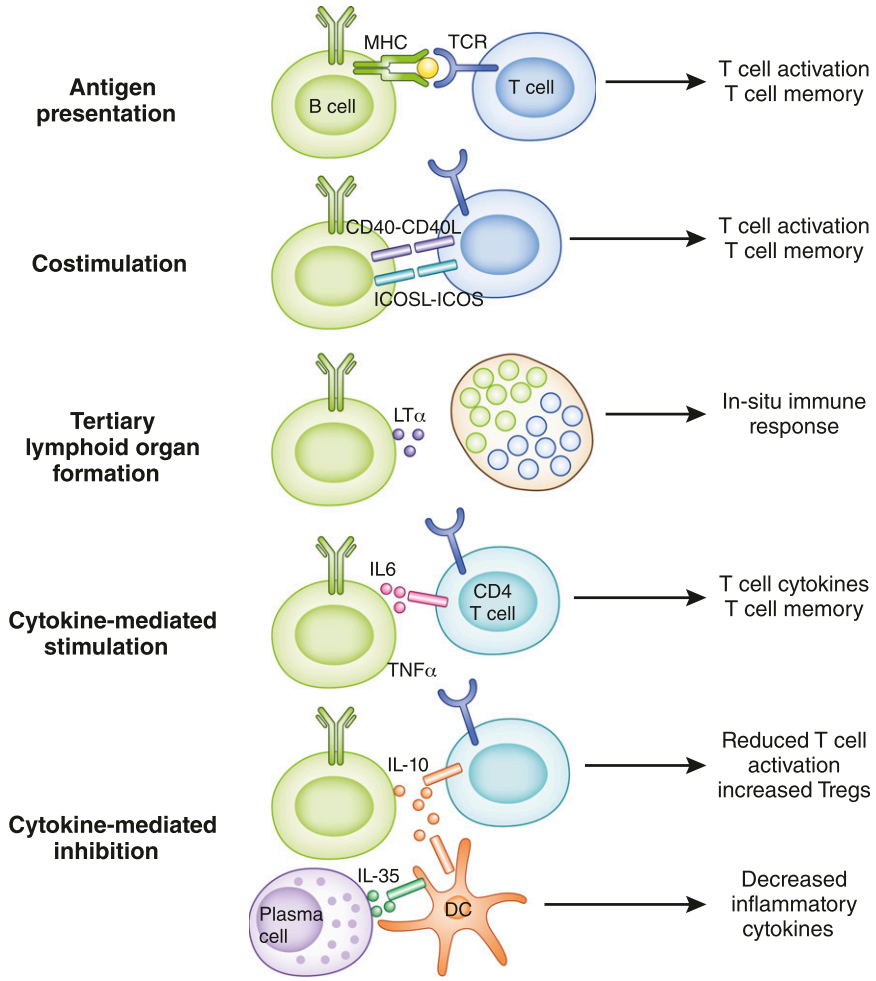

Figure 6. | **Cellular functions of B cells**. B cells interact with T cells and innate cells, such as dendritic cells (DCs), *via* several mechanisms that influence the outcome of the immune response. Antigen presentation, costimulation (such as CD40–CD40L, inducible costimulator ligand [ICOSL]–inducible costimulator [ICOS]), and cytokine production (such as IL-6 and TNF-α) contribute to enhanced T-cell activation and differentiation (*e.g.,* T follicular helper cells), cytokine polarization (*e.g.,* Th1 and Th17), and formation of long-lived memory T cells. Lymphotoxin (LTα) produced by B cells contributes to formation of tertiary lymphoid organs in peripheral tissues that are sites of *in situ* immune responses causing tissue injury. B cells and plasma cells also secrete cytokines, such as IL-10 and IL-35, that reduce T-cell activation and cytokine production and increase T cells with regulatory properties in addition to modulating functions of innate cells, such as DCs (*e.g.,* decreased IL-6 and IL-12), to attenuate immune responses.

boundary of the follicle adjacent to the T-cell zone (referred to as T–B border), where they initiate cognate interactions with early T follicular helper cells (Tfhs) (39,40). T-cell help for B cells comes in the form of costimulatory ligands (CD40L; inducible costimulator ligand) and cytokines (*e.g.*, IL-4, IL-21, and IFN-γ) that stimulate B-cell proliferation and differentiation.

Some of the activated B cells develop into extrafollicular plasmablasts and early memory B cells without entering the follicles (extrafollicular pathway). Activated B cells that upregulate *B-cell lymphoma 6* (*Bcl6*) return to the follicles (FO pathway), where they are retained by the expression of sphingosine-1-phosphate receptor 2 to form GCs with Tfhs, which support affinity maturation of immunoglobulin antigen–binding sites and immunoglobulin class switching (40,41).

Within the GC, IL-21 and costimulatory signals derived from Tfh (Figure 4) sustain extensive B-cell proliferation and induce gene expression programs essential for somatic hypermutation (SHM) and class-switch recombination (CSR) to generate high-affinity class-switched memory B cells and plasma cells (41). SHM and CSR require the expression of the DNA-editing enzyme activation-induced cytidine deaminase: SHM induces point mutations within the immunoglobulin gene segments that encode the variable antigen-binding regions, enabling selection of high-affinity clones into memory and plasma cell pools by competition for antigen within the GC; CSR replaces genes that determine isotype classes, allowing generation of antibodies with different effector functions without changing their antigen specificities (42–44).

GC B cells that have successfully acquired Tfh signals and competed for the limited antigen within the GC with high-affinity interactions upregulate *Bcl2* family prosurvival factors and are selected into the memory B-cell or plasma cell pools (37,45–47). Productive Tfh interactions with GC B cells initiate sequential expression of transcription factors, IFN regulatory factor 4, B lymphocyte–induced maturation protein 1 (also known as PR domain zinc finger protein 1), and X-box binding protein 1, which commit their differentiation into long-lived plasma cells after repression of *Bcl6* (48,49). *Blimp1* expression is essential for sustaining plasma cell development *via* both extrafollicular and FO pathways, while *Xbp1* functions to support immunoglobulin secretion (35,50–52).

Plasma cells home to the BM *via* C-X-C motif chemokine receptor 4, where they reside in survival niches supported by stromal cells secreting CXCL12 and cytokines (IL-6; APRIL) and produce antibodies maintaining serologic memory independent of further antigen exposure (35). Memory B cells recirculate and form extrafollicular or FO aggregates in lymphoid tissues, where they differentiate rapidly into plasma blasts (GC-dependent memory) or re-enter GCs upon antigen rechallenge (extrafollicular and GC-dependent memory), resulting in further diversified secondary antibody responses (47,53–56) (Figure 4). Memory B cells and plasma cells generate high-affinity immunoglobulin class-switched diversified antibodies, which are the basis of long-lived humoral immunity and are difficult therapeutic targets in autoimmune diseases.

At this juncture, it's relevant to discuss the two key cytokines, BAFF (also known as B-lymphocyte stimulator) and APRIL of the TNF family, required for survival of B cells during various stages from their initial development to terminal differentiation (Figure 5). BAFF and APRIL are produced by myeloid cells (such as DCs, macrophages, and neutrophils) and stromal cells (57,58) and bind to receptor transmembrane activator and cyclophilin ligand interactor (TACI) and B-cell maturation antigen, while BAFF also signals through B cell–activating factor-receptor (BAFF-R) (Figure 5) (57). BAFF is essential for survival and maturation of transitional B cells, sustains GC reaction, and supports CSR (57,59). Signaling through TACI, both BAFF and APRIL, promote T-independent antibody responses and CSR, while BAFF also functions in limiting B-cell expansion through TACI (57,60). Plasma cell survival requires APRIL and/or BAFF signaling through B-cell maturation antigen, whereas immunoglobulin class-switched memory B cells are maintained independent of BAFF or APRIL (57,61,62). Dysregulation of BAFF is associated with autoimmune diseases, such as SLE and ANCA-associated vasculitis (AAV), and targeting soluble BAFF using belimumab has shown benefit for patients with lupus nephritis (63–65).

B Lymphocytes as Enhancers and Regulators of Cellular Immunity

In addition to their obvious role in humoral immunity, it is now established that B lymphocytes contribute directly to cellular immunity *via* at least three mechanisms: (*1*) they serve as antigen-presenting cells (APCs) that enhance T lymphocyte–mediated immunity; (*2*) they function as *bona fide* cellular effectors that produce inflammatory cytokines; and (*3*) a subgroup of them, known as Bregs characterized by IL-10 secretion, modulate immune responses (Figure 6) (66,67). Moreover, B cells maintain secondary lymphoid organ architecture, particularly of the spleen, and promote the formation of ectopic lymphoid tissues (tertiary lymphoid tissues) at sites of chronic inflammation, which then become hot spots of local T- and B-lymphocyte activation (Figure 6) (68–72). Together, these "cellular" functions of B cells significantly contribute to the pathogenesis of autoimmunity and allograft rejection.

B Lymphocytes as APCs

Antigen captured by the BCR is internalized into endosomal compartments, where it is processed into peptides that then reemerge on the cell surface bound to MHC-1 and -2 molecules. This allows B lymphocytes to present antigenic peptides to both CD4 and CD8 lymphocytes as a "professional" APC (*e.g.*, a DC) would. In addition, and similar to DCs, B cells express the necessary costimulatory molecules and cytokines required for full activation of the T lymphocytes they engage. These include B7 and CD40 molecules, which ligate the T lymphocyte costimulatory receptors CD28 and CD40L, respectively, and the cytokines IL-6 and IFN-γ (73–75). B cells also express innate TLRs, which respond to pathogen-associated molecular patterns, further enhancing their APC function (75–77). Although on a per-cell basis B cells are not as potent APCs as DCs, the fact that they proliferate in response to antigen gives them a clear numeric advantage. Experimental data in mouse models of antimicrobial as well as lupus and antigraft immunity have established that antigen presentation

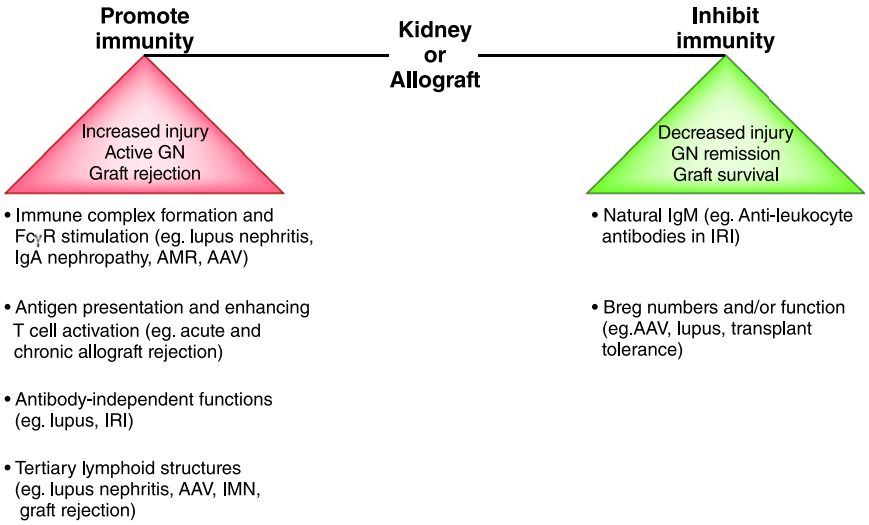

Figure 7. | **B cells as enhancers and regulators of immunity in kidney disease and transplantation**. B cells can promote or inhibit immune responses, mediating kidney injury, GN, and transplant rejection by various mechanisms of action, and the balance between these functions influences disease outcomes. Isotype-switched antibodies contribute to antibody-mediated rejection (AMR) and GN (*e.g.,* lupus, IgA nephropathy, ANCA-associated vasculitis [AAV]) by forming immune complexes and activating FcγR while natural IgM antileukocyte antibodies are protective in ischemia-reperfusion injury (IRI). Antibody-independent functions of B cells contribute to lupus and ischemia-reperfusion injury and mediate graft rejection by presenting antigen and driving T-cell activation. B cells form tertiary lymphoid structures that are the sites of local immune responses causing tissue injury in lupus nephritis, AAV, idiopathic membranous nephropathy (IMN) and graft rejection. Various B-cell populations with regulatory functions (*e.g.,* IL-10) contribute to graft survival and GN remission (*e.g.,* AAV), and their disrupted numbers or function are observed in transplant rejection and GN relapse (*e.g.,* AAV and lupus).

by B cells ensures optimal T-cell activation, cytokine production, and generation of long-lasting memory T cells (73,75,78–82). In the absence of this B-APC function, memory T-cell numbers and function are impaired after an antigenic challenge and organ damage is attenuated in lupus.

B Lymphocytes as Cellular Effectors

It is increasingly recognized that during an immune response, some B cells acquire the ability to produce effector cytokines with inflammatory properties (83). Examples of these are IFN-γ, TNF-α, and IL-17 (67,75,84,85). IFN-γ and TNF-α have direct injurious effects on endothelial and epithelial cells, thus contributing to both allograft rejection and inflammatory renal disease (86–89). Similarly, IL-17 stimulates cytokine and chemokine production by endothelia, epithelia, and fibroblasts, which then drive neutrophil infiltration and inflammation (90). Effector cytokines from B cells also influence activation of CD4 T cells, their cytokine production, and memory development (67,75,84,85), likely in a bystander fashion, unlike antigen presentation by B cells, which requires cognate interactions.

Bregs

A regulatory function for B cells has been demonstrated in multiple mouse models of autoimmunity and transplantation, whereby indiscriminate B-cell depletion or deficiency paradoxically caused worsening of disease outcomes (66,91–95). In each case, the regulatory function could be attributed to IL-10 production by a small subset of B cells. Overall, IL-10–producing Bregs constitute about 1% of all B lymphocytes in the mouse and appear to be present in all known major B-cell subpopulations (*e.g.,* FO and MZ B cells) (96). Recent data have shown that *T* cell *immunoglobulin mucin* 1 (TIM-1), a member of the T immunoglobulin and mucin domain family of proteins, serves as an inclusive marker for Breg (about 6%–8% of all B lymphocytes in the mouse express TIM-1 and about 30% of these produce IL-10) (96). A monoclonal antibody binding TIM-1 enhances allograft survival in a Breg-dependent manner. In addition to B cells, some plasmablasts (IgM^+CD138^+) exert regulatory functions *via* production of the cytokine IL-35 (97). *Via* IL-10 or IL-35, Bregs modulate innate cells, such as DCs, macrophages, or natural killer cells (*e.g.,* decreased IL-6 and IL-12), decrease inflammatory T-cell cytokines, and increase regulatory Tregs, curtailing the ongoing immune response (95,97–101). Bregs have also been identified in humans, specifically in the $CD24^{high}CD38^{high}$ transitional and $CD24^{high}CD27^+$ memory B-cell subsets, and their numbers correlate with better renal transplant outcomes (102–104). Altered numbers and/or function of Bregs have been described in SLE and AAV, contributing to disease pathogenesis and/or relapse (102,105–107). The presence of potent Bregs could explain why pan-depletion of B cells in humans using anti-CD20 (rituximab) has led to paradoxical or unsatisfactory clinical results in renal transplantation, as well as autoimmune renal disease (108,109). Similarly, indiscriminate use of antibodies targeting BAFF could be detrimental because Breg development and IL-10 production appear to depend on BAFF signaling through TACI (110,111). Selective agents that spare or enhance Bregs are therefore greatly needed to optimize B cell–targeting therapies.

Role of Antibodies and B Lymphocytes in Renal Disease

Role in Renal Transplantation

Interest in B lymphocytes and antibodies as causative agents in transplant rejection stems from the beginnings of renal transplantation, when it was realized that patients with preformed antibodies against donor antigens reject their grafts within minutes to hours after transplantation (so-called hyperacute rejection) (112). Preformed antibodies that cause hyperacute rejection are those against the ABO blood antigens or HLA. Careful ABO matching of donors and recipients and careful testing of the recipient's serum for antibodies against the donor's HLA before transplantation have eliminated hyperacute rejection in the clinic. However, the dilemma of what to do with prospective renal transplant recipients on the waiting list who are highly sensitized to the general population (*i.e.*, those with high panel-reactive antibodies) remained, many of them dying before a suitable donor could be identified. Strategies have therefore been devised to desensitize such patients, allowing them to receive a deceased- or living-donor kidney once their antidonor antibody titers had subsided. Successful strategies include the use of plasmapheresis and IVIG, the latter likely exerting its effects *via* Fc receptors by downmodulating B-cell function and Fab-mediated effects on target cells (23). Of note, ABO-incompatible heart transplantation, and possibly other organ transplantation, is feasible in infants before the development of significant anti-ABO antibody titers (113). Transplanted infants in fact acquire tolerance to the incompatible ABO antigen, providing firm proof that human B cells are prone to tolerance if challenged while the immune system is still relatively immature (114).

More recently, the significance of donor-specific antibodies (DSAs) that arise after transplantation has come to the fore. These antibodies, usually against donor HLA but sometimes directed against non-HLA epitopes, are associated with poor renal allograft outcomes because of acute or chronic antibody-mediated rejection (AMR) (115–119). AMR is often associated with acute or chronic cellular rejection and the presence of DSAs also correlates with increased risk of isolated cellular rejection, indicating that DSAs are a useful biomarker for heightened antidonor immunity (120–124). Strategies to combat the development of DSA have largely relied on the use of adequate T-lymphocyte immunosuppression, such as with tacrolimus, because pathophysiologic antidonor antibodies belong to T cell–dependent isotypes, usually complement-fixing (for example, IgG3) and the requisite role of T-cell help for B-cell differentiation. B-cell depletion with rituximab has been attempted as a prophylactic therapy at the time of renal transplantation (induction therapy) to improve graft outcomes or as a treatment for AMR (125–127). In the former case, it was paradoxically associated with increased, rather than decreased, risk of acute rejection and in the latter the results have been ambiguous (108,127).

These clinical studies highlight the heterogeneity of targeted B-lymphocyte populations (memory B cells, plasma cells) and functions (effector versus regulatory) and therefore the need to devise more selective depletional approaches. In addition, dysregulation of BAFF levels, especially after depletion of cells consuming BAFF, contribute to pathogenic antibody responses, indicating that pan-depletion of B cells can have deleterious effects on disease progression (128–131). Long-lived plasma cells lack CD20, the target of rituximab, and account for alloantibody production even after mature B cells that express CD20 are depleted. Targeting plasma cells using proteasome inhibitors alone has limited efficacy, likely due to rapid differentiation of memory B cells into plasma cells to repopulate depleted niches, underscoring the challenges in efficacious removal of pathogenic B cells (132).

Data emerging from experimental models and humans strongly suggest that B cells contribute to rejection independently of their antibody-producing role. In mice, B cells promote both acute and chronic rejection by functioning as APCs, and B-lymphocyte participation in the pathogenesis of chronic rejection can occur in the absence of secreted antibody (80,133). A recent study in human renal allograft recipients provided compelling evidence that although activation of B cells resulted in production of both TNF-α and IL-10, it was the relative abundance of TNF-α to IL-10 expression in transitional B cells that correlated strongly with acute rejection and 3-year graft outcomes (104). Patients with stable renal allograft function had similar numbers of transitional B lymphocytes and similar IL-10–to–TNF-α ratios as healthy individuals, while those with graft dysfunction had reduced transitional B-lymphocyte numbers and reduced IL-10–to–TNF-α ratios. B-lymphocyte clusters have also been observed within renal allografts undergoing acute and chronic rejection, contributing to local immune response and causing graft injury (134–137). Together, these findings underscore the importance of the cellular functions of B cells, whether regulatory or effector, in shaping renal allograft outcomes (Figure 7).

The role of B cells in renal transplantation tolerance has also been an intense area of study. Several independent reports provided evidence that operationally tolerant kidney transplant recipients (those who have stable graft function in the absence of all pharmacologic immunosuppression) exhibit various B-cell alterations within their peripheral blood mononuclear cells, including increased B-cell numbers, B cell–specific gene expression, transitional B cells producing IL-10, memory B cells with an inhibitory phenotype, and granzyme B–expressing B cells that curtail proliferation and cause apoptosis of CD4 effector T cells (138–142). These studies provide the impetus to explore new strategies to induce or enhance regulatory B cells in humans for the purpose of achieving tolerance or minimizing long-term, conventional immunosuppression after kidney transplantation.

Role in GN

B lymphocytes are incriminated in the pathogenesis of both systemic and kidney-targeted autoimmune diseases (Figure 7). They are responsible for the generation of autoantibodies and circulating immune complexes that deposit in the kidney and cause GN. As discussed previously, they can also contribute to tissue injury by producing inflammatory cytokines and by presenting antigen to T lymphocytes. The significance of B lymphocytes in human GN can be best inferred from studies that correlate circulating or renal interstitial B cell phenotype and function

with disease activity and from studies that attempted B-lymphocyte depletion or inhibition to treat the disease. These include SLE, AAV, Henoch-Schönlein purpura (HSP), cryoglobulinemia, and idiopathic membranous nephropathy (IMN).

Lupus Nephritis. SLE results from systemic loss of B-cell tolerance, leading to production of high titers of autoantibodies against double-stranded DNA (dsDNA), RNA, and nuclear proteins (143–145). Dysregulated BAFF levels and augmented signal transduction downstream of the BCR, specifically in the BtK-Lyn-Syk kinase pathway, contribute to increased B-cell activation with increased frequencies of memory and plasma cells in patients with SLE (65,131,144,146–148). The presence of anti-dsDNA antibodies identifies patients at risk of lupus nephritis, consistent with experimental evidence that these antibodies have a causative role in GN when deposited as immune complexes in the kidney. However, B cell–deficient but not antibody-deficient mice are protected from lupus nephritis, indicating that cellular functions of B cells also contribute to disease pathogenesis (149,150). In addition to glomerular lesions, lupus nephritis is characterized by inflammation and scarring of the renal interstitium, which predicts progression to renal failure.

Recent studies on human renal biopsy specimens have established the presence of conspicuous interstitial B lymphocyte infiltrates in lupus nephritis (151,152). These are often organized along with T cells and DCs into lymph node–like structures that are known as tertiary lymphoid tissues (151,153,154). B lymphocytes within these structures are actively dividing (supported by local BAFF and APRIL), are undergoing somatic hypermutation, and sometimes form germinal centers (151,153–155). The presence of germinal centers is strongly associated with tubular basement membrane immune complexes, providing a highly plausible link between local B-lymphocyte activation and progression of lupus nephritis in humans (151,153,154).

On the basis of the causal relationship between B-lymphocyte activation and SLE, B lymphocyte–targeting therapies have been tested in the clinic in patients with SLE who have or do not have lupus nephritis. First tested were the monoclonal anti-CD20 antibodies, rituximab and ocrelizumab, which target the B-lymphocyte surface molecule CD20 expressed on mature B cells, causing depletion of these cells. Two large randomized phase 3 trials failed to demonstrate statistically significant superiority of B-cell depletion with either agent over standard-of-care therapy in patients with active proliferative lupus nephritis (156,157). However, a trend toward more patients reaching complete or partial remission at 1 year (primary endpoint), and improved proteinuria and renal function (secondary endpoints) was observed in the B-lymphocyte depletion groups (109,156,157). Patients treated for class 3 lupus nephritis attained complete remission most successfully, while those with class 5 lupus nephritis were the least likely to respond (109). It is unclear whether adjunct therapies, such as those also targeting T cells, would improve response rates because T cells can contribute to B-cell activation and mediate tissue damage in SLE (158). Favorable outcomes with rituximab treatment were associated with attaining complete B-cell depletion, reconstitution of predominantly naive and immature B cells, and sustained suppression of memory B cells and plasma cells, whereas changes in anti-dsDNA antibodies did not correlate with response (159–161). Development of antichimeric antibodies against rituximab and elevated BAFF levels with poor B-cell repopulation were associated with lack of response (109,130,162).

Belimumab is a monoclonal IgG1 humanized antibody against soluble BAFF and is the first biologic therapy to be approved by the US Food and Drug Administration for SLE in 50 years. Depletion of B cells by BAFF deprivation using belimumab normalized complement levels and reduced dsDNA titers and SLE severity in two phase 3 randomized clinical trials (163). *Post hoc* analysis of the trial data demonstrated improvement in renal flare rates and reduction in proteinuria with anti-BAFF, with greatest benefit in those with high disease activity, suggesting efficacy in lupus nephritis, while serologic memory to past vaccine immunizations was preserved (163–165). It remains to be examined whether the beneficial effects of anti-BAFF in lupus are due to resetting aberrant checkpoints of peripheral B-cell tolerance eliminating autoreactive clones and/or attenuating T-cell activation by blocking costimulatory functions of BAFF (65,166).

In contrast to anti-BAFF, treatment with atacicept, a recombinant fusion protein that blocks both BAFF (membrane-bound and soluble) and APRIL, led to severe hypogammaglobulinemia with serious infections and worsening proteinuria when given with mycophenolate mofetil in lupus nephritis (60).

Taken together, these studies confirm the key requirement for APRIL and not BAFF in maintaining serologic memory (60,165). Small molecule inhibitors of Btk and Syk have shown early promise in mouse models of lupus nephritis and await examination of efficacy in patients with lupus nephritis (167).

AAV. AAV comprises systemic syndromes characterized by necrotizing inflammation of blood vessels; the most significant clinicopathologic manifestation in the kidney is rapidly progressive GN (168). AAV is characterized by circulating ANCA, the principal targets of which are proteinase 3 and myeloperoxidase present in neutrophils and monocytes. In mouse models, the transfer of antimyeloperoxidase antibodies or B cells from affected animals transfers disease to healthy animals (169). In humans, B-lymphocyte clusters have been observed in rapidly progressive GN kidneys, similar to those described in lupus nephritis (170). Aberrations in circulating B cells have been described in patients with active disease in AAV with increased BCR signaling, altered proportions of CD5+ B cells, and decreased Breg numbers or function (105–107,171). Not surprisingly, therefore, rituximab in combination with corticosteroids is an effective (and Food and Drug Administration–approved) therapy for inducing remission in patients with AAV, but its efficacy in treating patients with advanced renal disease has not been established yet (172). Rituximab has also been successfully used to treat relapsing or refractory AAV and to maintain remission, and neither extent of B-cell depletion nor ANCA titers correlate consistently with response (173). The observation that circulating levels of BAFF correlate with disease activity has prompted an ongoing phase 3 trial to test belimumab combined with

azathioprine in the maintenance of remission in patients with AAV (63,173–175). Recent studies suggest an inverse relationship between circulating Bregs in the peripheral blood and disease activity or relapse in patients with AAV, highlighting the need to better understand the role of these important regulatory cells in controlling autoimmunity (105–107,171).

HSP. HSP represents a spectrum of IgA nephropathy with multiorgan involvement and vasculitis of small vessels. Immune complexes formed with aberrantly glycosylated IgA1 cause vasculitis that affects the kidney in approximately 50% of patients with HSP, with similar renal lesions as in IgA nephropathy. CD5-expressing B1 B cells are increased in patients with IgA nephropathy and are the source of galactose-deficient IgA, which contributes to disease pathogenesis (9). Although anecdotal reports have shown a significant response to rituximab in patients with HSP who did not respond to conventional therapy, further studies are needed to examine the use of B-cell depletion in treating HSP (176–178).

Cryoglobulinemia. Polyclonal IgG with or without monoclonal IgM forms immune complex deposits, causing vasculitis and renal disease in patients with mixed cryoglobulinemia. Hepatitis C virus (HCV) infection is the main cause of mixed cryoglobulinemic vasculitis, and the most common renal lesion is membranoproliferative GN. HCV-related MZ B-cell expansion and aberrant activation-induced cytidine deaminase expression sustains B-cell activation and immunoglobulin production (179,180). Rituximab treatment of HCV-associated mixed cryoglobulinemic vasculitis is superior to conventional therapy, supporting a key role for B cells in disease pathogenesis (181,182).

Idiopathic Membranous Nephropathy. Subepithelial deposition of IgG in the glomerular capillary wall is a hallmark of idiopathic membranous nephropathy and, along with the presence of B cells in renal biopsy specimens, implicates B lymphocytes in the pathogenesis of the disease (183). Moreover, approximately 80% of patients have antibodies against the podocyte-derived antigen, phospholipase A2 receptor (PLA2R) (184). Circulating levels of anti-PLA2R antibodies are a biomarker for disease activity and response to treatment (185). Single-arm studies suggest a role for rituximab in treating idiopathic membranous nephropathy; however, responses are detected in only about 60% of patients, with others progressing to ESRD (186). An ongoing phase 3 randomized trial is comparing efficacy of rituximab to cyclosporine in inducing long-term remission in idiopathic membranous nephropathy (187). Results of another phase 2 open-label clinical trial testing the efficacy of belimumab in PLA2R autoantibody-positive idiopathic membranous nephropathy on remission of proteinuria and autoantibodies are awaited (ClinicalTrials.gov NCT01610492). An important need is development of immunologic or other biomarkers to help identify patients who are likely to successfully respond to B-lymphocyte depletion.

Role in AKI

It is increasingly recognized that immune cells play an important role in the pathogenesis of AKI caused by sepsis, ischemia, or toxins. Recent studies identify the contribution of B cells and antibodies in renal ischemia-reperfusion injury (IRI). Following IRI, B cells infiltrate the kidney and interfere with the repair phase, and in their absence injury is attenuated with increased tubular proliferation (188,189). Conversely, adoptive transfer of serum recapitulated renal injury and transfer of B cells worsened tubular atrophy (188,189). B1 B cells infiltrated kidneys undergoing IRI, and reduction of peritoneal B cells only partially attenuated IRI; this finding suggests that other B-cell lineages, such as FO and MZ B cells, could also contribute to injury (189,190). Natural IgM enriched in antileukocyte autoantibodies protected against IRI by markedly attenuating leukocyte infiltration and activation of pathogenic T cells (191). Thus, early antibodies produced by B cells, such as natural IgM, play a protective role, while their later antibody responses and/or cellular functions could be pathogenic in IRI.

Concluding Remarks

B cells link the innate and adaptive arms of immune response by their ability to respond rapidly to damage-associated molecular patterns and antigenic stimuli, and also form long-lived serologic memory. B cells perform diverse functions, such as antibody secretion, cytokine production, antigen presentation, and lymphoid architecture organization, that intersect with both innate (such as DCs) and adaptive T-cell roles in shaping the outcome of the immune response toward immunity or tolerance (Figures 6 and 7). Disruption of B-cell tolerance by cell-intrinsic (BCR signaling) or cell-extrinsic (BAFF, T-cell help) defects, dysregulated BAFF levels, and impaired regulatory functions contribute to pathogenesis of autoimmunity. Depleting B cells could therefore potentially re-establish B-cell tolerance by purging autoreactive clones, eliminate pathogenic antibody-producing B cells, and interrupt cellular functions of B cells that enhance pathogenic T cell activation. However, nonselective pan-depletion of B cells can also remove the beneficial Bregs, increase BAFF levels, and potentially worsen disease. Instead, targeting B cells to correct specific defects or remove pathogenic B cells while sparing others would prevent undesired immune activation or immune deficiency. Future studies aimed at disease-specific understanding of how pathogenic B cells arise could facilitate not only the development of novel selective therapies but also the optimal use of existing therapies, such as rituximab and belimumab, for best outcomes.

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Disclosures

None.

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Immunosuppressive Medications

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Abstract

Immunosuppressive agents are commonly used in the nephrologist's practice in the treatment of autoimmune and immune-mediated diseases and transplantation, and they are investigational in the treatment of AKI and ESRD. Drug development has been rapid over the past decades as mechanisms of the immune response have been better defined both by serendipity (the discovery of agents with immunosuppressive activity that led to greater understanding of the immune response) and through mechanistic study (the study of immune deficiencies and autoimmune diseases and the critical pathways or mutations that contribute to disease). Toxicities of early immunosuppressive agents, such as corticosteroids, azathioprine, and cyclophosphamide, stimulated intense investigation for agents with more specificity and less harmful effects. Because the mechanisms of the immune response were better delineated over the past 30 years, this specialty is now bestowed with a multitude of therapeutic options that have reduced rejection rates and improved graft survival in kidney transplantation, provided alternatives to cytotoxic therapy in immune-mediated diseases, and opened new opportunities for intervention in diseases both common (AKI) and rare (atypical hemolytic syndrome). Rather than summarizing clinical indications and clinical trials for all currently available immunosuppressive medications, the purpose of this review is to place these agents into mechanistic context together with a brief discussion of unique features of development and use that are of interest to the nephrologist.

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Introduction

Immunosuppressive agents have a long history, with a recent acceleration in growth in number. After the discovery by Nobel Prize awardee Philip Hench that the corticosteroid cortisone had significant anti-inflammatory effects in patients with rheumatoid arthritis (RA) in 1949 (1) and the independent discoveries by Calne *et al.* (2), Murray *et al.* (3), and Zukoski *et al.* (4) that azathioprine (AZA) was an effective immunosuppressive agent in the prevention of kidney allograft rejection in the early 1960s (2–4), many of the mechanisms of the immune response remained opaque. The 1960s and 1970s were marked by a borrowing of cyclophosphamide from the developing field of cancer chemotherapy for use in immune diseases and transplantation, whereas the use of antilymphocyte serum as a lymphocyte-depleting agent gained favor in the developing field of kidney transplantation. The late 1970s and early 1980s brought revolutionary changes in drug development and discovery; two key developments were the technology to develop monoclonal antibodies (mAbs) for human therapeutic use and the discovery of the immunosuppressive effects of cyclosporin A from fermentation extracts of the fungal species *Tolypocladium inflatum* (5,6). The 1990s were a period of significant immunosuppressive drug development, because increased insight into B and T cell development, activation, and proliferation, cytokine and chemokine signaling, and complement activation led to targeted therapeutics, particularly mAbs that could later be humanized (Figure 1). In reciprocal fashion, drug discovery often led to further understanding of the mechanisms of the immune response. Similar to cyclosporin A, sirolimus (previously called rapamycin) was discovered and developed as an antifungal, but it was found to have antineoplastic and immunosuppressive properties, the mechanisms of which were only later appreciated and described as mammalian target of rapamycin (mTOR) pathways (7,8).

In recent decades, immunosuppressive drug development has slowed from its accelerated pace in the late 1990s, but it still shows steady growth. With improvements in efficacy and specificity of existing agents, it is increasingly difficult to develop an agent that meets superiority and safety measures necessary to gain regulatory and public opinion approval. This is particularly true for diseases that the nephrologist may encounter: most uses of immunosuppressive agents are in rare, orphan category diseases that are difficult or unlikely to be studied in large multicenter trials. Thus, many of the newer agents that the nephrologist may encounter will inevitably be in off-label use stemming from experience in other fields, such as rheumatology and oncology. Exceptions to this generalization are emerging attempts to treat the inflammation identified in the settings of AKI and maintenance hemodialysis.

To provide a framework for understanding the multitude of immunosuppressive agents currently available and in late-stage development, this review will summarize key agents commonly encountered in nephrology practice by immune cell target rather than disease state or clinical indication. Together with the previous reviews within this Renal Immunology

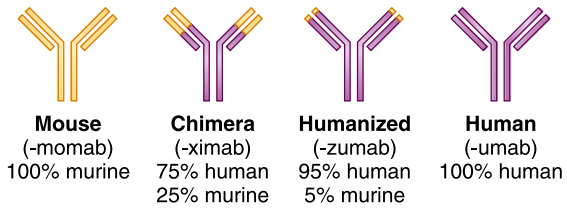

Figure 1. | Schematic representation and nomenclature of mAbs in clinical use. The suffix denotes of the degree of human versus non-human components.

Series, it is hoped that the reader will be able to intertwine the science with its clinical applications.

T Cell–Directed Therapy

Therapeutic agents that target T cell function can be separated into those that inhibit signal 1 (the interaction of the T cell receptor [TCR] complex with an antigen-presenting cell [APC] either carrying antigen or in the case of transplantation, acting as antigen itself) and its resulting intracellular signaling and those that inhibit signal 2 (the costimulatory signal provided by additional T cell/APC interaction that leads to full activation of the T cell) (Figure 2). Agents that inhibit further downstream activation and proliferation (occasionally referred to as signal 3) are typically driven by cytokine production and signaling and will be discussed in later sections.

Agents Targeting Signal 1

Anti-TCR Agents. Inhibition of the first point of antigen presentation (the MHC/TCR complex) has been an attractive target in transplant immunosuppression. The murine anti-CD3 mAb Muromonab-CD3 (OKT3) was the first mAb approved as a drug for human use in 1986 for the prevention of rejection in renal, heart, and liver transplants (9). It targeted the CD3 subunit of the TCR complex and led to rapid elimination of functional T cells. It is now no longer in production because of waning utilization, primarily because of significant side effects related to the mitogenicity associated with its murine source. This early experience led to the development of humanized forms of anti-TCR–based agents in an effort to reduce this mitogenicity as well as other anti-TCR mAbs that targeted other receptor subunits (10–12). These next generation therapeutics were subsequently forwarded for the treatment of new-onset diabetes and as induction agents in kidney transplantation but have been hindered by ongoing safety and efficacy issues.

Calcineurin Inhibitors (Cyclosporin and Tacrolimus). After initial TCR binding, a calcineurin-dependent signaling pathway is induced that leads to initial T cell gene transcription necessary for additional activation. Two commonly used calcineurin inhibitors (CNIs; cyclosporin and tacrolimus) and one investigational agent (voclosporin) inhibit the ability of calcineurin to dephosphorylate nuclear factor (NF) of activated T cells (NFAT), required for translocation from cytoplasm to nucleus, and prevent calcineurin-dependent gene transcription (13,14). In the early 1980s, cyclosporin transformed the field of

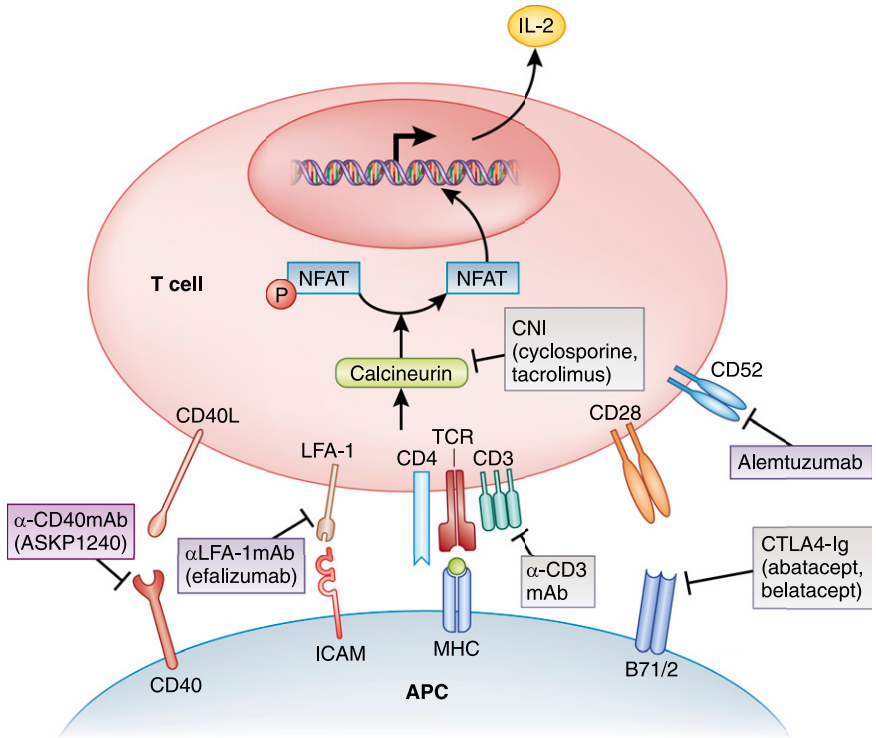

Figure 2. | Immunosuppressive agents targeting T cell/antigen-presenting cell interaction and early T cell activation. This depicts agents that inhibit signal 1 and signal 2 in T cell activation. APC, antigen-presenting cell; CNI, calcineurin inhibitor; CTLA4, cytotoxic T lymphocyte–associated protein 4; ICAM, intracellular adhesion molecule 1; LFA-1, lymphocyte function–associated antigen-1; NFAT, nuclear factor of activated T cells; TCR, T cell receptor.

transplantation with dramatic reductions in acute rejection rates, and it has been shown to be effective in a number of immune diseases, including a number of glomerulopathies (15,16). In the late 1990s, tacrolimus was introduced in kidney transplantation and over time, has shown to be more potent in reducing the rates of acute rejection (17,18). Growing experience in glomerular disease suggests its use to be of similar value as cyclosporin (19). One potential nonimmunosuppressive mechanism that could explain CNI efficacy in glomerular disease is the ability to inhibit synaptopodin degradation in the podocyte, thereby stabilizing the actin cytoskeleton and reducing proteinuria (20).

Tacrolimus and cyclosporin share the side effect of CNI-induced vascular constriction that contributes to an increase in BP as well as diminished renal perfusion. This is primarily a dose-dependent phenomenon; it can result in renal ischemia and acute tubular necrosis in the acute setting, and with prolonged ischemia, it can result in chronic kidney injury. Aside from the direct vascular effect on renal blood flow, the potential direct nephrotoxic effects of CNI agents remain an active area of debate and research (21). To address these issues of toxicity and side effect profiles (including post-transplant diabetes), alternative formulations of tacrolimus (extended release) as well as the novel CNI voclosporin have been developed and are approved or in late-phase clinical trials in transplantation (22–24).

Agents Targeting Signal 2

Costimulation Blockade by CD80/86:CD28 Targeting (Abatacept and Belatacept). The interaction of CD80/86 on the APC with CD28 on the T cell (costimulation) is required for optimal T cell activation. After upregulation and the generation of an effective immune response, the T cell expresses the cell surface molecule cytotoxic T lymphocyte–associated protein 4 (CTLA4), which competitively binds to CD80/86 and downregulates the T cell response. To mimic this downregulatory effect, human IgG heavy chains were linked with CTLA4 to create a fusion protein for clinical use. The first generation CTLA4-Ig that was clinically developed, abatacept, is approved for the treatment of RA and is under investigation in other autoimmune diseases, including lupus (25). Recently, abatacept has been proposed to be of potential value in the treatment of FSGS in a small series of cases, in which immunostaining of podocytes is positive for CD80 (B7–1) (26). Abatacept was ineffective in preclinical primate studies in the prevention of kidney transplant rejection, leading to development of another CTLA4-Ig with significantly higher affinity for CD80/86 for transplantation (belatacept) (27). This agent is Food and Drug Administration (FDA) indicated for use as a substitute for CNIs at the time of transplant (28).

Costimulation Blockade by CD154:CD40 Targeting (Anti-CD40 mAb). The CD154 (also known as CD40L; present on activated T cells): CD40 (on APCs) interaction is a critical step in T cell costimulatory signaling, because this interaction leads to the upregulation of CD80/86 on APCs. Targeting the induced surface molecule CD154 on activated T cells was a focus of drug development until it was recognized that CD154 was also present on platelets, and agents binding this cell surface molecule led to an increase in thrombotic events in both primate and early-phase human trials (29). Attention has, thus, turned to targeting CD40. A number of mAbs against CD40 are in development, with a fully human anti-CD40 (ASKP1240; Astellas) under study in phase 2 clinical trials in kidney transplantation (30).

B Cell–Directed Therapy

The goals of B cell inhibition include inhibiting not only the humoral response to auto- or alloantigen but also, the APC function and B/T cell interactions that lead to efficient T cell activation and proliferation. B cell therapies can be considered in the context of the agents that inhibit maturation and differentiation of the resting B cell throughout its development to a highly active antibody-producing plasma cell (Figure 3).

B Cell Targeting

Anti-CD20 Targeting: Rituximab, Ocrelizumab, Ofatumumab, and Veltuzumab. CD20 is a transmembrane protein present on pre-B and mature B lymphocytes, but it is not present on stem cells, normal plasma cells, or other cell lines. Its role in B cell development includes regulation of activation for cell cycling and B cell differentiation. The first agent to target CD20, rituximab, is a chimeric anti-CD20 mAb (30% murine and 70% human) that leads to B cell depletion through a number of mechanisms, including complement-dependent cytotoxicity, growth arrest, and apoptosis (31). This depletion is durable, with B cell counts suppressed for up to 6–9 months and occasionally, beyond. Efficacy data pertinent to nephrology practice include the treatment of ANCA-associated vasculitis, with supportive data in antibody-mediated rejection and forms of nephrotic syndrome (32–34). The chimeric nature of the antibody leads to side effects attributable to cytokine release, such as fever, bronchospasm, and hypotension. Agents that are humanized (ocrelizumab) or fully humanized (ofatumumab) have been developed to minimize these untoward infusion reactions. However, ocrelizumab development in RA has been discontinued because of an increased risk of serious infections (35). A phase 1/2 trial of ofatumumab in RA has shown preliminary efficacy, with mild/moderate infusion reactions still prevalent (36). All anti-CD20 therapy carries a risk of hepatitis B reactivation in patients positive for hepatitis B surface antigen or hepatitis B core antibody (37). Therefore, before starting treatment, patients should be screened for hepatitis B surface antigen and hepatitis B core antibody.

Anti-CD22 Targeting: Epratuzumab. CD22 is expressed on B cells during B cell maturation and loss of CD20 expression. B cell receptor signaling is modulated by phosphorylation of CD22, which regulates B cell activation. Epratuzumab is a humanized anti-CD22 mAb that inhibits B cell activation and has a more modest depleting effect on B cells than rituximab. It is currently in phase 3 trials in patients with moderate to severe SLE after phase 2 trials suggested a low rate of adverse events, similar to placebo (38,39).

Targeting B Cell Differentiation: Belimumab and Atacicept

A key pathway for differentiation of B cells is the binding of the cytokine B cell–activating factor (BAFF; also referred to BlyS) to its B cell receptors [(BAFF-R, B cell maturation (BCMA), and transmembrane activator and CAML interactor (TACI)] and the binding of the cytokine proliferation–inducing ligand to its B cell receptors (BCMA and TACI).

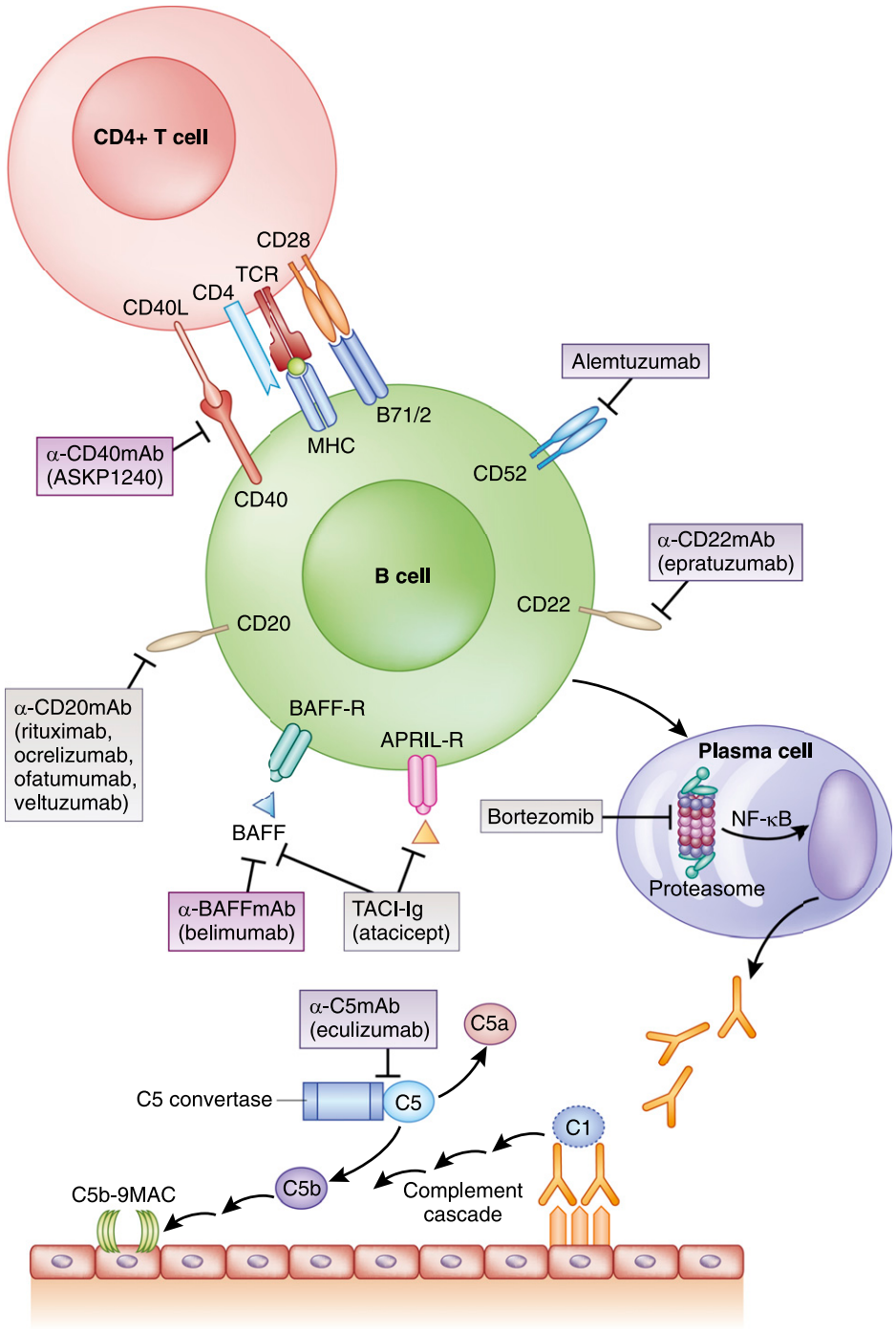

Figure 3. | **Immunosuppressive agents targeting T cell/B cell interaction, plasma cell function, and complement-mediated injury.** APRIL, proliferation-inducing ligand; BAFF, B cell–activating factor; MAC, membrane attack complex; TACI, transmembrane activator and CAML interactor; TCR, T cell receptor.

These interactions lead to increases in NF-$\kappa\beta$, which in turn, promote B cell differentiation and inhibit apoptosis. Belimumab is a humanized anti-BAFF/BlyS mAb that interferes with ligand/receptor binding and inhibits this maturation. It is currently approved for use in active SLE (40). However, patients with severe active lupus nephritis were excluded from the pivotal trials, and *post hoc* analyses of renal outcomes were inconclusive in showing improvement in clinically relevant renal outcomes (41). Atacicept is a recombinant fusion protein that inhibits both BlyS and proliferation-inducing ligand. In phase 2 trials in RA, efficacy was not shown (42), whereas in a phase 2/3 trial in patients with lupus nephritis, the trial was terminated after pronounced reduction in Ig levels and the occurrence of severe pneumonia, leaving in question the further development of this agent (43).

Plasma Cell Targeting: Bortezomib

All B cell agents previously described have no direct activity against plasma cells, and thus, for diseases in which plasma cell maturation and antibody production are felt to be a primary pathogenic mechanism, these previous agents are expected to have limited efficacy. Critical to the function of highly metabolic cells, such as plasma cells, is the ability to regulate the degradation of proteins through the proteasome, which is present in all eukaryotic cells. Inhibition of the proteasome leads to inhibition of cell cycling and induction of apoptosis (44). Bortezomib is a proteasome inhibitor that was found to be particularly effective in treatment of the plasma cell dyscrasia multiple myeloma and indicated by the FDA for the treatment of advanced myeloma in 2003 (45). Subsequent studies showed a benefit in myeloma with renal involvement and antibody-mediated rejection of kidney allografts by targeting antibody-producing plasma cells (46,47). Side effects of peripheral neuropathy, cytopenias, and gastrointestinal effects occur in a dose-dependent fashion.

Complement Inhibition (Eculizumab)

The role of complement in renal disease is increasingly appreciated and contributes to disease by either direct activation of complement or initial antibody fixation and subsequent activation of complement. There is direct evidence of its role in the thrombotic microangiopathic changes seen in atypical hemolytic uremic syndrome (aHUS), antibody-mediated injury of the kidney allograft, and C3 GN (previously called dense deposit disease or membranoproliferative GN type 2) (48–50). To date, one agent is available for clinical use. Eculizumab is a humanized mAb to C5 that effectively inhibits its cleavage to C5a and C5b. Because C5a is a neutrophil chemoattractant and because C5b is required to form the C5b-9 membrane attack complex, inhibition of this enzymatic step results in blockade of proinflammatory, prothrombotic, and lytic functions of complement (Figure 3). Its efficacy is most apparent for cases of aHUS in which a complement factor mutation has been identified. However, therapy is recommended in all patients with aHUS who are at risk for (or suffering from) renal failure given the potential of an unidentified complement mutation as a contributing factor (51,52). Although data regarding the use of eculizumab for the treatment of antibody-mediated rejection are currently at the level of case reports, its use pretransplant for the prevention of antibody-mediated rejection as part of a desensitization protocol has been shown to be of benefit (53). The cost of eculizumab remains a significant barrier to use. Inhibition of the complement cascade increases the risk of serious infection from encapsulated bacteria; thus, vaccination for *Neisseria* meningitis, *Streptococcus pneumonia*, and *Haemophilus* influenza type b should be performed before therapy.

Additional indications for complement inhibition may be in the treatment or prevention of ischemia/reperfusion injury (AKI in the native kidney and delayed graft function in the transplanted kidney) (54,55). A multicenter trial investigating the use of eculizumab in the prevention of delayed graft function is underway, and numerous compounds targeting the complement pathway are in preclinical investigation.

Agents Targeting Cytokines

Cytokines are proteins that are secreted by a variety of cell types and function to direct the initiation, differentiation, and up- and downregulation of the immune response. Pharmacologic targeting of specific cytokines is expected to redirect or inhibit an untoward immune response (Figure 4).

Nonspecific Cytokine Inhibition

Corticosteroids. Corticosteroids bind to the intracellular glucocorticoid receptor and modulate a multitude of cellular functions by binding to glucocorticoid-responsive elements in the nucleus. Effects on the immune system are also numerous but most clearly related to inhibition of all cytokine transcription by blocking transcription factors, such as NF-$\kappa\beta$ and activator protein-1 (56). This has numerous downstream effects, such as (*1*) depletion of T cells because of inhibition of IL-2, inhibition of Th1 differentiation, and induction of apoptosis, (*2*) eosinophil apoptosis (either directly or by inhibition of IL-5), and (*3*) macrophage dysfunction because of inhibition of IL-1 and TNF-α. Its effect on neutrophil function is modest; however, neutrophil migration to sites of inflammation is impaired, bone marrow secretion of neutrophils is increased, and apoptosis is decreased, all of which contribute to leukocytosis (57). Similarly, B cells are not significantly inhibited by corticosteroids, with only mild decreases in Ig production. Its side effect profile is well appreciated clinically and frequently maligned as a chronic therapy (58). Thus, despite its efficacy in a wide range of immune and inflammatory conditions, a primary focus of many clinical development programs and clinical trials is to find agents with similar efficacy but greater specificity in immunosuppression without the attendant side effects of corticosteroids.

Janus Kinase Inhibition (Tofacitinib). Janus kinases are cytoplasmic tyrosine kinases that mediate signaling from cytokine receptors to phosphorylation of signal transducers and activators of transcription, enabling signal transducers and activators of transcription to enter the nucleus and regulate gene expression and transcription. An inhibitor of Janus kinase, tofacitinib, inhibits cytokine receptor signaling from a number of cytokines, including IL-2, -4, -7, -9, -15, and -21, and has shown efficacy in psoriatic arthritis and RA (59). Its development as an alternative for CNI in kidney transplantation was halted after a phase 2b trial showed similar rejection rates, better GFR, lower rates of post-transplant diabetes, and higher rates of cytomegalovirus and BK virus infection and post-transplant lymphoproliferative disease (60).

Specific Cytokine Inhibition

IL-2 Receptor Antagonist (Basiliximab). Activated T cells produce IL-2 and express the α-subunit of the IL-2 receptor, rendering it fully functional. After T cells become activated in response to signals 1 and 2 activation, IL-2 binding and subsequent intracellular signaling lead to proliferation of T cells. Humanized antibodies to the α-subunit of the IL-2 receptor (IL-2 receptor antagonists basiliximab and daclizumab) limit proliferation of activated T cells and have been approved for the prevention of acute rejection in kidney transplantation (the latter is no longer in production), primarily in patients with lower immunologic risk (61).

Targeting TNF-α. TNF-α is an acute-phase cytokine released by macrophages, T cells, B cells, neutrophils, natural killer cells, mast cells, and some nonimmune cell types

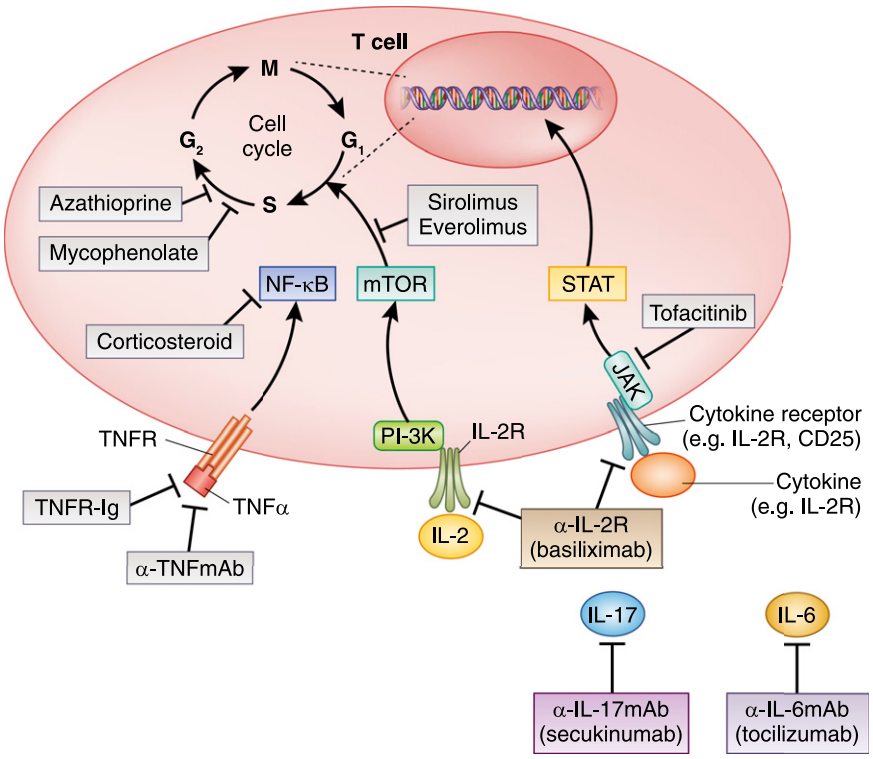

Figure 4. | **Immunosuppressive agents targeting later stages of T cell differentiation and proliferation and selected cytokines.** This depicts agents that inhibit signal 3, T cell proliferation. JAK, Janus kinase; mTOR, mammalian target of rapamycin; PI-3K, phosphoinositide 3-kinase; R, receptor; STAT, signal transducer and activator of transcription.

(smooth muscle and epithelial cells) in response to tissue injury (62). Well described roles for TNF-α include its release by Th1 T cells to promote ongoing expansion of Th1 cells and proinflammatory responses and its release by synovial macrophages to induce synovial cells to increase collagenase production, thus promoting bone and joint destruction. TNF-α binds to its receptors (TNF receptor 1 [TNFR1] and TNFR2) and stimulates apoptotic pathways as well as NF-$\kappa\beta$ signaling, which partially explains its diverse physiologic effects. Currently, there are five TNF inhibitors clinically available and indicated for the treatment of a variety of rheumatic diseases. Infliximab, adalimumab, golimumab, and certolizumab are mAbs to TNF-α that differ in the degree of chimerism and route of administration (intravenous versus subcutaneous) (63). Etanercept is a TNFR fusion protein bound to IgG. All carry the risk of increased susceptibility to intracellular pathogens, such as tuberculosis, coccidiomycosis, and *Cryptococcus* (64).

IL-1 Inhibition (Anikinra, Rilonacept, and Canakinumab). IL-1 is a signature proinflammatory cytokine and a primary effector of many inflammatory conditions, including RA and adult-onset Still's disease. Two isoforms have been identified: IL-1α and IL-1β. IL-1α is constitutively expressed, present intracellularly in vascular endothelium, mucosal epithelium, keratinocytes, liver, lung, and kidney, and released on cell necrosis (*e.g.*, from injury/ischemia). IL-1β is induced by macrophage/monocytes in response to TNF and other proinflammatory cytokines (65). Inhibition of IL-1 has been an attractive target not only for states of dysregulated inflammation but also, in the mitigation of injury in response to ischemic events, including postmyocardial infarction. At present, three agents are clinically available: anikinra (an IL-1 receptor antagonist), rilonacept (a soluble decoy receptor), and canakinumab (an anti–IL-1β mAb). Of interest, IL-1β is elevated in patients on maintenance hemodialysis and may contribute to the chronic inflammation noted in this population. Preliminary studies have examined the feasibility of targeted inhibition of IL-1 in patients on chronic hemodialysis, with additional studies ongoing (66).

IL-6 Inhibition (Tocilizumab). IL-6 is expressed in response to inflammatory stimuli and contributes to CD8 T cell differentiation, B cell differentiation, and activation of the hepatic acute-phase response. Increased circulating IL-6 has been associated with mortality in AKI and ESRD, malnutrition in ESRD, and rejection in recipients of kidney transplants (67). The humanized mAb tocilizumab is an inhibitor of IL-6 and has shown efficacy in RA (68). A phase 1/2 study in highly sensitized patients awaiting kidney transplantation (NCT01594424) is currently evaluating safety and effect on donor-specific anti-HLA antibodies, with other kidney transplant studies planned (NCT02108600), but currently, no studies in native acute or chronic renal disease are forthcoming.

IL-17 Inhibition (Secukinumab). IL-17 is a cytokine produced by CD4 T cells, but it is also secreted by CD8 T cells, eosinophils, monocytes, and neutrophils. IL-17 functions to increase inflammatory cell migration by stimulating chemokine release, and it increases APC activity to enhance adaptive immune responses. IL-17 has been shown to be a key mediator of injury in a number of autoimmune diseases,

including ankylosing spondylitis, psoriasis, and multiple sclerosis (MS). A human anti–IL-17A mAb (secukinumab) has been developed for clinical use, and recently, two phase 3 trials in psoriasis now show greater efficacy compared with placebo or the TNF-α inhibitor etanercept (69).

Agents Targeting Chemokines and Cell Adhesion

At present, there are a handful of agents that target specific chemokines or their receptors that have been approved for clinical use, although a multitude of others have been tested in early clinical development (70). Difficulties in successful drug development targeting chemokines can be attributed to poorly predictive preclinical models, a redundancy in chemokine signaling that circumvents targeted therapy, and an incomplete understanding of the signals that up- or downregulate chemokines that can occasionally appear paradoxical. Those agents that have found clinical applicability reflect this diversity. For example, approved chemokine receptor antagonists include the CCR5 receptor antagonist maraviroc used in the treatment of HIV, the CXCR4 antagonist plerixafor approved for hematopoietic stem cell mobilization, and the CCR4 humanized mAb mogamulizumab approved for the treatment of T cell lymphoma. Relevant to nephrology practice, emapticap is an inhibitor to CCL2 (also known as monocyte chemotactic protein 1), which has been studied in phase 1 and 2 trials in diabetic nephropathy, with proof-of-concept studies showing a reduction in albuminuria presumably by inhibiting inflammatory cell migration into the kidney (71).

Antibodies targeting adhesion molecules have had a circuitous and tenuous route to clinical use. The agent FTY720 (fingolimod) is a sphingosine 1-phosphate (S1P) receptor modulator that binds to S1P receptors on lymphocytes, preventing lymphocyte migration from lymph node to the vasculature. Clinical trials in kidney transplantation were halted after no improvement over mycophenolate was noted together with untoward side effects of prolonged QT interval, bradycardia (caused by S1P receptor binding on cardiomyocytes), and macular edema. However, S1P receptors are present on neural cells and seem to be critical in neural cell migration during central inflammation in MS; fingolimod has now gained approval for the treatment of relapsing/remitting MS (72). Similarly, efalizumab is a humanized mAb to leukocyte function–associated antigen-1 that prevents lymphocyte activation and cell migration from the vasculature into tissues. It had been shown to be effective in moderate/severe plaque psoriasis, islet transplantation, and kidney transplant, but it has been withdrawn from the United States market after high rates of post-transplant lymphoproliferative disease and brain infections, including progressive multifocal leukoencephalopathy, were reported (73,74). Finally, natalizumab, a humanized mAb against the adhesion molecule α-4 integrin, blocks lymphocyte migration from vasculature to tissues and was approved by the FDA in 2004 for MS and later, Crohn's disease; however, similar to efalizumab, there have been concerns regarding serious infections, such as progressive multifocal leukoencephalopathy, and it has been withdrawn and returned to the market, with strict monitoring programs in place (75).

Pooled Polyclonal Antibodies as Immunosuppressive Agents

Immune Globulins

Intravenous Ig. Intravenous Ig (IVIG) is an Ig extract pooled from several thousand plasma donors to create a product that is IgG rich. Although IVIG was initially used to provide passive immunity in patients with immune deficiencies (with doses of 500 mg/kg monthly), ongoing experience and research suggest a very diverse immunomodulatory and anti-inflammatory role of IVIG therapy noted with high-dose therapy (1–2 g/kg) (76). Although the mechanisms underlying these effects are broad and may differ in each disease state in which a benefit has been reported, a few common mechanisms often cited include (*1*) direct binding to natural antibodies, immunomodulatory proteins (*e.g.*, cytokines), or superantigens and pathogens, (*2*) inhibition of complement fixation on target tissues by acting as a complement sink, (*3*) Fc receptor (FcR) binding and subsequent inhibition of the FcR-mediated recycling of native IgG, and (*4*) stimulation of FcR-induced anti-inflammatory pathways. Its use in the nephrology specialties is primarily in the setting of kidney transplant for desensitization (inhibition and elimination of preformed HLA or blood group (ABO) antibodies to achieve a negative cross-match and permit transplant) and treatment of antibody-mediated rejection (77). Although effective as monotherapy, the addition of other modalities, including plasmapheresis, rituximab, and bortezomib, provides greater immunomodulatory effects and improved clinical outcomes (78). There are different products available that differ in their concentration of IgG, stabilizers, osmolality, and IgA content. The latter is important in that rare patients who suffer from severe IgA deficiency may produce anti-IgA antibodies and suffer anaphylactic reactions when receiving IVIG products. Side effects of IVIG include infusion-related effects (including hives, fever, and anaphylactoid reactions), headaches (including aseptic meningitis), thrombotic complications (including myocardial infarction), and AKI (predominantly seen with sucrose-containing preparations) (79).

Polyclonal Antithymocyte Globulin. Therapeutic antibodies to human lymphocyte antigens have been created by a number of techniques: by immunizing rabbits with human thymocytes (Thymoglobulin), immunizing horses with human thymocytes (Atgam), or immunizing rabbits with lymphocytes from a Jurkat T cell leukemia line (Fresenius antithymocyte globulin [ATG]). The resulting IgG fraction from sera is then purified and pasteurized for use. The resulting antibodies are polyclonal (*i.e.*, all cell surface molecules presented on the infused thymocytes may lead to a humoral response in the immunized source, and the final preparation contains a vast array of diverse antibodies to various antigens). Although the Igs in these cases are anti–T cell predominant, there are many shared cell surface antigens among T cells and other immune cells; thus, the ATG products also have activity against B cells, monocytes, and neutrophils to lesser degrees. The primary mechanism of action of ATGs is lymphocyte depletion, predominantly by complement-dependent lysis and T cell activation–induced apoptosis (80). The two rabbit ATG products are most widely used at present for the treatment and prevention of acute kidney allograft rejection (81). When compiling small head-to-head trials of

Thymoglobulin versus ATG Fresenius, Thymoglobulin may be considered more potent in terms of both efficacy and untoward effects (82). All ATG products, as nonhumanized Igs, are prone to symptoms consistent with cytokine release (fever, chills, hypotension, and pulmonary edema) related to natural killer cell and macrophage/monocyte binding of FcR binding as well as cellular cytotoxicity (83).

Immunosuppressive Agents with Multiple Cellular Targets

Panlymphocyte Depleting Agents: Anti-CD52 (Alemtuzumab)

Anti-CD52 (Campath 1H and alemtuzumab) is a humanized mAb that binds to CD52, an antigen of unclear physiologic significance that is present on both B and T cells. Ligation of CD52 results in depletion of both lymphoid cell lines. Its ability to induce prolonged, significant lymphopenia for up to 6–12 months after dosing led to its use in refractory chronic lymphocytic leukemia (84). As a humanized antibody, fewer infusion-related side effects are noted than with other depleting agents, such as ATG. In kidney transplantation, growth in off-label use as an induction agent had grown over the last decade but recently, was abruptly diminished subsequent to manufacturer removal from the United States market in preparation for relabeling for use in MS (85). Kidney transplant trials suggest equivalence to other depleting agents in the prevention of rejection and efficacy in corticosteroid-withdrawal regimens, but the long-term effect of prolonged lymphopenia on the risk for infection or post-transplant lymphoproliferative disorder is not determined (86,87).

Antiproliferative Agents: mTOR Inhibitors (Sirolimus and Everolimus)

In lymphoid cells, the mTOR pathway leads to cell cycle progression from G1 to S phase and proliferation in response to cytokine stimulation, including but not limited to IL-2 receptor binding (Figure 4). Inhibitors of mTOR that are clinically available include sirolimus, everolimus, and temsirolimus; the primary immunosuppressive mechanism of action of these agents has been attributed largely to inhibition of lymphocyte proliferation (7). However, mTOR signaling is not isolated to lymphocytes, and this intracellular signaling pathway has been described in monocytes/macrophages, dendritic cells, natural killer cells, and endothelial cells (8). Thus, inhibition of mTOR may be expected to lead to a number of clinically relevant effects related to its antiproliferative, antiviral, anti-inflammatory, and antitumor effects as well as a diverse side effect profile (88). mTOR inhibitors have been evaluated for their ability to inhibit cyst growth in autosomal dominant polycystic kidney disease, with conflicting and modest results in large multicenter trials (89,90), have been effective in reducing intimal proliferation and obliterative vasculopathy in heart transplantation (91), have shown efficacy in the treatment of angiomyolipomas (92), and have been approved for use in the treatment of advanced renal cell, breast, and other malignancies (93,94).

Antimetabolites: Inhibition of DNA Synthesis

AZA. AZA is an analog of 6-mercaptopurine; the metabolites of these agents act as both purine analogs (interfering with *de novo* purine synthesis and thus, DNA and RNA synthesis) and immunomodulatory agents (contributing to S-G2 cell cycle arrest in addition to other anti-inflammatory effects) (95). Toxicities that are often noted include bone marrow suppression and gastrointestinal intolerance (primarily upper gastrointestinal symptomatology). The xanthine oxidase inhibitors allopurinol and febuxostat slow elimination of 6-mercaptopurine and exacerbate the risk of these side effects (96). Use of AZA has dramatically decreased in kidney transplantation and rheumatologic diseases with the introduction of mycophenolate (discussed below), except in the setting of pregnancy planning. AZA has not been associated with teratogenicity, unlike mycophenolate (97).

Mycophenolate. Mycophenolate is an inhibitor of IMPDH, the rate-limiting enzyme of guanine nucleotide synthesis critical for *de novo* purine synthesis and thus, DNA synthesis. T cells (and B cells) are dependent on the *de novo* pathway for DNA synthesis. Similar to AZA, primary side effects are gastrointestinal and hematopoetic. Its efficacy in the prevention of rejection compared with AZA together with better tolerability than mTOR inhibitors have led to its use as the primary antimetabolite in transplantation, despite a lack of definitive long-term data showing improved graft outcomes. Its efficacy in autoimmune diseases and other glomerular diseases is increasingly appreciated (98,99). Therapeutic drug monitoring has not revealed a clear relationship between mycophenolate exposure and prevention of rejection in recipients of kidney transplants or clinical efficacy parameters in rheumatologic diseases (100). Two formulations are available, mycophenolate mofetil and enteric-coated mycophenolate sodium, with generic formulations available for both. Despite generic availability, cost still is significantly greater than AZA.

Leflunomide. Leflunomide is a pyrimidine antagonist that blocks DNA synthesis and cell cycling from S to G2 phase. Its specific mechanism of action entails inhibition of the key rate–limiting enzyme dihydro-orotate dehydrogenase critical for *de novo* pyrimidine synthesis (101). It is approved for use in RA, but its *in vitro* activity against cytomegalovirus and BK virus has prompted its off-label use in kidney transplantation for its potential dual antiviral and anti-inflammatory properties (102). A very long half-life (>14 days), hepatic and bone marrow toxicities, and a lack of compelling data supporting an advantage over reduction in immunosuppression alone in the clinical management of BK virus have reduced interest in leflunomide for this purpose, but it still is used off label in circumstances of drug-resistant cytomegalovirus infection (103).

Cytotoxic Agents (Cyclophosphamide) as Immunosuppressive Agents

A brief mention of cyclophosphamide is necessary given its use as an immunosuppressant in life-threatening or severe rheumatologic and renal diseases, including ANCA-related vasculitis, lupus nephritis, and other systemic vasculidites. Cyclophosphamide is an alkylating agent that is toxic to all human cells to differing degrees, with hematopoetic cells forming a particularly sensitive target (104). Primary toxicities, such as bladder toxicity, gonadal toxicity, and later malignancy, have led to attempts to minimize exposure (<250–300 mg/kg cumulative dose to avoid gonadal

toxicity and <360 mg/kg cumulative dose to minimize the risk of malignancy), attempts to use intermittent intravenous rather than daily oral therapy to minimize exposure, and search for alternative agents (for example, mycophenolate mofetil in lupus nephritis and rituxan in ANCA-related vasculitis) (105–107).

Disclosures

A.C.W. has served as a consultant for Astellas, Tolera, and Veloxis and currently receives research/grant support from Alexion, Bristol Meyer Squibb, and Novartis.

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