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

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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+ cell expressing CD25, the IL-2 receptor-1, cells were described and distinguished 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.

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 autoimmune disease, 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 autoimmune 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 ε-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).

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 adrenocorticotrophic 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 thymocyte 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 TCRαβ T cells is effector programmed to become soldiers. A minority of peripheral CD4+ TCRαβ 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
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, 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.

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 by 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 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 other leukocytes but not T cells. The intracytoplasmic domain of CD45 describes and glycosylated in a gp220 expressed by B cells and

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

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

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 TCRAβ 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-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
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 lineage-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 CD127hi 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−CD127hiFOXP3− 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

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**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.
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 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 cytokines, IL-2, 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 Th17 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.

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).

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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 lacks the inflammatory activity of M1 cells and Ig-secreting plasma cells. Tfhs are regulated by B cells, thereby promoting their maturation to memory B cells and Tfh cells interact with 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–CD127hiFOXP3+ T cells become homeostatic in B cells, and their expression of CD40 on follicular and Tfh cells does not express perforin and granzyme. Th2 cytokines induce CD4+CD25+FOXP3+ Tcells to revert to effector T cells (79,80). Homeostatic regulation ensures lineage (78), and therefore, they and their progeny cannot be switched off and stabilize 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 <1% 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 CD127hi T 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 Tfh cells 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 binding to B7.1 and B7.2 blocks the activation of effector T cells. Thus, both in vivo and in vitro 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.

Activation of nTregs

The most important professional Tregs are CD4+CD25+CD127hiFOXP3+ 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–CD127hiFOXP3+ 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). Tregs 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 <1% 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 CD127hi T 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 Tfh cells from periphery-induced iTregs.
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 (57,58,59). 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 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 Th2 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 ×1:1. We found that treatment with IL-5 promoted these cells to control autoimmunity (65) and transplant rejection (57).

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 ×1:1. We found that treatment with IL-5 promoted these cells to control autoimmunity (65) and transplant rejection (57).

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 scurvy 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).
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 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.

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
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 iTregs. iTregs are distinct from iTregs and Tr1 cells and are FOXP3 (138). iTregs 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

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−iTregs (pathway 5). AICD, activation induced cell death.
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 a 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 is Th2 and Th1 responses.

There is a T cell and macrophage interstitial infiltrate in the kidney in acute ischemia (155), with ureretic 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 reaction 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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