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The T_{reg}/Th17 Cell Balance: A New Paradigm for Autoimmunity

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ABSTRACT: Regulatory T cells and T helper 17 cells are two recently described lymphocyte subsets with opposing actions. In this review, we discuss the mechanisms that promote development of these cells from common precursors and the specific factors that impact their cell numbers and function. Altered regulation of this key developmental checkpoint may contribute to the pathophysiology of autoimmune diseases by tipping the balance toward inflammation. We also present recent findings that suggest how the equilibrium between regulatory T cells and proinflammatory T helper subsets might be pharmacologically restored for therapeutic benefit. **(*Pediatr Res* 65: 26R–31R, 2009)**

The recent discovery of two novel subsets of $CD4^+$ T lymphocytes has led to a paradigm shift in the understanding of how autoimmune responses are both mediated and regulated. One of these cell subsets, $CD4^+$ T helper 17 (Th17) lymphocytes, is a key effector cell in rodent models of human diseases including collagen arthritis and experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. A second $CD4^+$ T lymphocyte subset, termed regulatory T (T_{reg}) cells, is essential for dominant immunologic tolerance. Surprisingly, both Th17 and T_{reg} cells can develop from naïve $CD4^+$ T cell precursors under the influence of the same cytokine, transforming growth factor β1 (TGFβ1). In this review, we discuss the ontogeny of T_{reg} and Th17 cells, as well as their known immune functions. We present the hypothesis that certain forms of autoimmunity may result when $CD4^+$ T cell differentiation is biased away from T_{reg} cells and toward the Th17 cell phenotype. Finally, we discuss ways that the Th17/T_{reg} cell balance might be modified to restore immune homeostasis, resulting in therapeutic benefit in autoimmune conditions.

Regulatory T Lymphocytes

$CD4^+$ lymphocytes with suppressor properties have been known to exist for many years. However, the central role of $CD4^+$ $CD25^+$ T_{reg} cells expressing the transcription factor forkhead box protein 3 (Foxp3) in immune regulation was initially disclosed by studies involving the inbred Scurfy mouse strain. In these animals, frameshift mutations in Foxp3 transmitted by X-linked inheritance result in a fatal syndrome of extensive immune activation, leading to oversecretion of numerous cytokines and multiorgan infiltration by inflammatory cells (1). Orthologous mutations were shown to be responsible for the human condition immune dysregulation-polyendocrinopathy-enteropathy-X-linked (2–4). Foxp3 gene transfer to $CD4^+$ $CD25^-$ T cells confers suppressive properties (5–7), confirming that Foxp3 in these cells is sufficient to suppress proliferation and cytokine secretion by effector Th cells. Although Foxp3 itself may not specify T_{reg} cell lineage commitment (8,9), maintenance of the regulatory phenotype in thymically derived T_{reg} cells requires constitutive Foxp3 expression (10). Moreover, depletion of T_{reg} cells in adult or neonatal mice induces the Scurfy phenotype, whereas adoptive transfer of relatively small numbers of Foxp3-expressing T_{reg} to these animals provides complete protection from disease (11). Taken together, these experiments emphasize the dominant role of T_{reg} cells in maintaining immunologic tolerance throughout life.

Foxp3-expressing T_{reg} cells exist in at least two forms. Antigen-specific "natural" T_{reg} (nT_{reg}) cells develop as a distinct lineage in the thymus, from where they are exported as a cell type dedicated to maintaining self-tolerance (12). The nT_{reg} cells derived in the thymus are anergic *in vitro* (13) but exhibit proliferation at steady state *in vivo* (14). These cells express the high-affinity form of the interleukin-2 (IL-2) receptor but depend on an exogenous source of IL-2 to maintain Foxp3 expression (15).

A second type of $Foxp3^+$ $CD4^+$ lymphocyte, termed "induced" regulatory T (iT_{reg}) cell, is not formed in the thymus. Rather, iT_{reg} cells differentiate from mature naïve $CD4^+$ T cells in peripheral lymphoid organs and other tissues upon cellular activation in the presence of TGFβ1 (16,17). In contrast to nT_{reg} cells, the iT_{reg} cell phenotype seems to be less stable. Thus, iT_{reg} cells constitute of a dynamic pool of $CD4^+$ T cells capable of acquiring and losing Foxp3 expression, based on the regulatory needs of the host (18,19). Under certain conditions *in vivo*, $Foxp3^+$ $CD4^+$ iT_{reg} cells at mucosal surfaces can undergo epigenetic DNA changes, which result in a more stable iT_{reg} cell phenotype (20). The respective contributions of nT_{reg} and iT_{reg} cells to tolerance remain incompletely defined. Possibilities include complementary roles based on overlapping but nonredundant functions, or simple additive models based largely on cell numbers. In some model systems, iT_{reg} cells are preferentially induced in the mesenteric lymph nodes and in the lamina propria of the

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Abbreviations: FoxP3, forkhead box protein 3; **EAE,** experimental autoimmune encephalomyelitis; **iT_{reg} cell,** induced regulatory T cell; **RORγt,** retinoic-acid-related orphan receptor γt; **Th17 cell,** T helper 17 cell; **T_{reg} cell,** regulatory T cell

intestine, and may be particularly important at mucosal surfaces, where TGFβ1 is abundant. Localized stores of retinoic acid promote this process (21–23). Other Foxp3$^-$ IL-10-producing CD4$^+$ T lymphocytes with regulatory properties have been described (24,25).

TGFβ1 does not seem to be required for differentiation of nT_{reg} cells in the thymus, but it does plays a role in nT_{reg} cell function by supporting their survival in the periphery. This role of TGFβ1 in nT_{reg} cell survival is highlighted by observations in TGFβ1 and conditional T cell-specific TGF-β receptor II knockout mice, which develop a lethal syndrome resembling the Scurfy phenotype during the neonatal period. Intrathymic development of nT_{reg} cells in these animals seems to proceed normally, whereas survival of Foxp3$^+$ cells in peripheral lymphoid tissues is impaired (26–30). However, early thymic development of nT_{reg} cells is deficient in mice lacking the TGFβ receptor I, suggesting that the thymus produces another cytokine that substitutes for TGFβ (31).

Both nT_{reg} and iT_{reg} cells have been shown to suppress immune effector cells by a variety of cell contact dependent and independent mechanisms. These include the production of cytokines such as IL-10 and IL-35, sequestration of cytokines essential for cell growth such and IL-2, surface expression of the immunosuppressive molecule cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and utilization of the perforin-granzyme pathway to kill activated targets or tumor cells (32–39). In addition, T_{reg} cells may influence immune responses indirectly by modulating dendritic cell function (40). It seems plausible that the mechanisms of iT_{reg} and nT_{reg} cell function may be nonredundant.

Th17 Cells

Before the elucidation of Th17 cells as a unique CD4$^+$ T lymphocyte subset, it was believed that fully differentiated effector CD4$^+$ T cells existed in two forms: Th1 cells, the effectors of cell-mediated immunity, and Th2 cells, which promote humoral immune responses (41). The signature cytokine of Th17 cells, IL-17A (formerly IL-17), was first identified by subtraction hybridization experiments (42), and was later shown to induce joint damage in murine models of arthritis (43). It was subsequently proposed that IL-17-secreting CD4$^+$ T cells might represent a distinct lineage (44). This hypothesis was recently confirmed (45,46). The biology of Th17 CD4$^+$ lymphocytes has been the subject of several recent reviews (47–51).

Th17 cells are derived from naïve CD4$^+$ precursor cells and secrete a characteristic profile of cytokines including IL-17A, IL-17F, IL-21, and IL-22. Th17 cells have been shown to have a role in immunity to extracellular pathogens. However, the considerable interest that has been generated surrounding these cells is a result of studies challenging the dogma that most organ-specific autoimmune diseases are Th1-mediated. In EAE (52) and collagen-induced arthritis (53), deletion of the p19 chain of IL-23, a cytokine critical for Th17 cell growth, results in protection from disease and specific absence of Th17 cells. In contrast, inhibition of Th1 cell differentiation *via* mutation of the IL-12 receptor does not. These findings point to a unique role for Th17 cells in organ-specific autoimmune disease.

Upon binding to their respective receptors, Th17 cytokines exhibit a variety of proinflammatory effects. IL-17A, and the related cytokines IL-17B-E, are capable of binding to the receptor IL-17RA receptor, which is expressed on a variety of cell types, including hematopoeitic cells, fibroblasts, endothelial, and epithelial cells. Receptor engagement results in expression of proinflammatory chemokines, and this effect is further enhanced in the presence of other proinflammatory cytokines such as tumor necrosis factor alpha (47,54). IL-21 acts in an autocrine fashion to promote Th17 cell growth and differentiation and has effects on humoral immunity (55,56). Among its effects, IL-22 promotes dermal acanthosis and has an important role in a mouse model of psoriasis (57).

TGFβ1 and Th17 Cell Differentiation

In view of the established role of TGFβ1 in T_{reg} cell biology, the finding that TGFβ1 is also essential for Th17 differentiation in mice was of considerable interest. Three groups demonstrated that culture of naïve, CD4$^+$ murine lymphocytes in the presence of low concentrations of TGFβ and the proinflammatory cytokine IL-6 results in acquisition of the Th17 cell phenotype (58–60). The role of TGFβ in human Th17 cell differentiation has been controversial. However, recent studies (61–63) have shown that TGFβ is required for Th17 differentiation from naïve CD4+ T cells, albeit in minute amounts. IL-23, IL-21, and the proinflammatory cytokines IL-1 and IL-6 also promote human Th17 cell growth and differentiation.

An explanation of the two possible fates of a naïve CD4$^+$ T cell after exposure to TGFβ1 requires an understanding of the transcription factors involved in Th17 cell differentiation. Exposure of a naïve CD4$^+$ T cell to TGFβ1 and IL-6 results in induction of RORγt, a orphan retinoic acid nuclear receptor, that directs Th17-specific differentiation (64). The effects of IL-6 are mediated at least in part by STAT3 (65), which induces RORγt (66). In mice, another retinoic acid receptor, RORα, has been shown to act synergistically with RORγt to promote Th17 cell differentiation (67).

The divergent fates of a CD4$^+$ lymphocyte after exposure to TGFβ1 are accounted for, at least in part, by molecular antagonism that occurs between the transcription factors specific for these cell types, RORγt and Foxp3 (63,68,69). This interaction, whereby Foxp3 inactivates RORγt function, involves the Foxp3 exon-2 encoded sequence and does not require nuclear translocation of Foxp3 or association with DNA (18). A similar interaction has been shown to occur between Foxp3 and the Th17-specific transcription factor RORα (70). These findings do not preclude the possibility that Foxp3 affects Th17 differentiation in additional ways *via* transcriptional effects, as other studies have reported (18,71).

It is important to note that certain cytokines antagonize Th17 cell differentiation. For example, IL-2 is a potent inducer of Foxp3, and also inhibits Th17 cell differentiation *via* a STAT5-dependent mechanism (72). Cytokines associated with Th1 or Th2 cell differentiation, including IL-4, IL-12, and interferon-γ, direct naïve CD4$^+$ lymphocytes toward

other lineages. The dendritic cell-derived cytokine IL-27 is a potent inhibitor of Th17 cell differentiation (73,74). Cell activation in the presence of this cytokine and TGFβ1 results in generation of the Tr1 cell phenotype (75,76), which can confer protection against murine EAE (77).

Although Th17 cell differentiation seems to be fundamentally similar in humans and mice, there may be some important interspecies differences. Whereas most murine naïve $CD4^+$ T cells have the potential to develop into Th17 effector cells, in humans this potential seems to be restricted to a small subset of naïve $CD4^+$ cells bearing the cell surface marker CD161 (78).

Studies examining the effects of T_{reg} cells on Th17 cell differentiation have shown mixed results. In coculture experiments, iT_{reg} cells do not retard Th17 cell differentiation from naïve $CD4^+$ T cell precursors, and may even augment Th17 cell differentiation in the presence of IL-6, through a contact-dependent mechanism involving expression of TGFβ1 on their cell surface (79). However, these observations do not necessarily reflect the effects of iT_{reg} cells *in vivo*, because adoptive transfer of these cells results in amelioration of inflammation in a mouse model of colitis, accompanied by a reduction in numbers of mucosal IL-17-producing T cells (22). On balance, it seems that once the iT_{reg} cell differentiation pathway gains ascendancy, iT_{reg} cells are likely to retard generation of Th17 cells at mucosal surfaces.

Immune Deviation in the iT_{reg}/Th17 Pathway: A Novel Approach to Treatment of Autoimmune Disease?

The identification of Th17 cells as effectors of tissue-specific autoimmunity has led to a flurry of scientific activity aimed at inhibiting these cells and their secreted products. An intriguing alternative approach involves pharmacologically altering iT_{reg} and Th17 cell differentiation or expansion, using cytokines, cytokine inhibitors, and small molecule inhibitors of key signaling pathways. There has been progress in this rapidly evolving field.

Proinflammatory cytokine antagonists. The *in vitro* Th17-promoting effects of IL-1 and IL-6 on human Th17 cell differentiation and growth suggest that antagonists of these cytokines might retard Th17 differentiation *in vivo* and promote differentiation of naïve $CD4^+$ cells toward regulatory cell pathways. *In vivo* evidence supporting the role of IL-6 antagonism on Th17 cell differentiation is provided by a study in EAE, in which administration of neutralizing anti-IL-6 antibodies resulted in inhibition of disease and reduced numbers of myelin oligodendrocyte glycoprotein peptide-specific Th17 and Th1 cells (80). In this study, increased numbers of peptide specific, $Foxp3^+$ $CD4^+$ cells were not observed. In a different report, mice genetically deficient in the naturally occurring IL-1 inhibitor IL-1 receptor antagonist IL-1RA were found to develop destructive arthritis, associated with expansion of Th17 cells. This effect was found to be indirect and dependent on IL-23 (81).

No studies examining the effects of antagonists of IL-1 or IL-6 on Th17 or iT_{reg} cell differentiation in humans have been reported. However, Tocilizumab, a humanized MAb directed against IL-6Rα, has been used in Japan for treatment of Castleman's disease since 2005 (82), and phase III studies of its use in rheumatoid arthritis (83,84) and juvenile systemic idiopathic arthritis (85) have been completed. Anakinra, a soluble recombinant IL-1 receptor antagonist is already approved for treatment of systemic forms of arthritis. It will be of interest to examine Th17 and iT_{reg} cell populations in patients treated with these medications. Interestingly, human therapeutic use of infliximab, a MAb against the proinflammatory cytokine tumor necrosis factor alpha results in increased numbers of iT_{reg} cells in patients with rheumatoid arthritis (86,87) and Crohn's disease (87). The mechanism of this effect is unclear but may be mediated by dendritic cells.

Interleukin-2. T_{reg} cells express a high affinity form of the IL-2 receptor, consisting of three chains: a β chain (CD122), a common γ chain, and an α chain (CD25). Binding of IL-2 to its receptor on T_{reg} cells has been shown to enhance expression of Foxp3 *via* a STAT-5 dependent pathway, and promote cell survival (72). Humans with genetic mutations in CD25 suffer from a syndrome of immune dysregulation resembling immune dysregulation-polyendocrinopathy-enteropathy-X-linked, further emphasizing the importance of this receptor complex for maintaining normal T_{reg} cell function (88,89). However, in addition to its effects on T_{reg} cells, IL-2 also promotes the growth of other T lymphocytes that express a lower affinity form of the IL-2 receptor, consisting of a β and a γ chain but lacking a α chain. A monoclonal anti-mouse IL-2 antibody that blocks binding of IL-2 to the lower affinity form of its receptor but allows for binding of this cytokine to its high-affinity receptor *via* a putative exposed epitope was recently reported (90). Thus, IL-2/anti-IL-2 immune complexes containing this antibody allow for selective stimulation of T_{reg} cells. In a subsequent report, IL-2-containing immune complexes were administered to nonobese diabetic mice, which suffer from a form of autoimmune diabetes mellitus. This treatment resulted in increased numbers of T_{reg} cells in inflamed pancreatic islets, and protection from diabetes (91). Selective stimulation of T_{reg} cells *via* the high-affinity IL-2 receptor represents an exciting concept in treatment of autoimmunity. However, application of this concept to humans would require development of an anti-IL2 antibody with properties similar to that used in murine studies.

Retinoic acid. In humans, vitamin A is absorbed from the diet. Its principal physiologically significant metabolite *in vivo* is the trans isomer of retinoic acid, all-trans retinoic acid (ATRA) (92). Recent studies have shown that ATRA markedly influences the fate of naïve T cells activated in the presence of TGFβ1. ATRA inhibits Th17 cell differentiation, and promotes Foxp3 expression *in vitro*. This effect occurs independent of IL-2 and STAT5 (93), is potent enough to override the Th17-promoting effects of IL-6, and is associated with down-regulation of the Th17 cell-specific transcription factor RORγt. Moreover, iT_{reg} cells generated *in vitro* by cell activation in the presence of TGFβ1 and ATRA were effective at preventing disease in a mouse model of colitis, whereas

iT_{reg} generated in the presence of TGFβ1 alone were only partially protective (22).

These recent studies provide insight into how an essential dietary component may exert its effects in part by contributing to a salutary balance between iT_{reg} cells and Th17 immune effector cells. The World Health Organization has recommended dietary vitamin A supplementation, due in part to the possible beneficial effects of vitamin A on innate and acquired immune function. Maintenance of mucosal tolerance is one plausible way by which vitamin A could also exert beneficial immune effects. Together, these data suggest the possibility an additional approach to treatment of autoimmune disease, by alteration of the iT_{reg}/Th17 cell balance using pharmacologic ATRA analogues.

Type I interferons. Interferon β1 has been used clinically to treat multiple sclerosis for several years, even though the mechanisms of action have not been well understood. Shinohara et al (94) showed that engagement of the Type I interferon receptor on dendritic cells resulted in IL-27 secretion, and suppression of Th17 cell generation in coculture experiments. This effect was mediated through inhibition of an intracellular phosphoprotein, osteopontin. In an EAE model, mice whose dendritic cells were deficient in osteopontin, displayed higher serum concentrations of IL-27, fewer IL-17$^+$ cells (but more interferon γ^+ cells) in lymph nodes, and delayed onset of EAE. These findings provide a possible mechanism whereby interferon-β1 has been used beneficially to treat multiple sclerosis (95). By extension, they support further investigation of IL-27 itself for treatment of Th17 cell-mediated autoimmune diseases.

Environmental toxins. The aryl hydrocarbon receptor (AhR) is a transcription factor best known as the ligand for the toxin dioxin. However, it also binds to other toxins and to endogenous ligands. Using gene expression profile analysis, murine Th17 cells were shown to express the AhR. Activation of AhR by addition of an exogenous toxin resulted in increased expression of IL-17 and IL-22 by Th17 cells, and enhanced pathology in an EAE model. IL-22 expression was abrogated and EAE was ameliorated in AhR-deficient mice (96). In a different study, the AhR was shown to directly regulate Foxp3 expression *via* a mechanism involving modulation of TGFβ signaling. Depending on which AhR ligand was used, opposite effects on T_{reg} cell and Th17 cell differentiation were observed. Whereas dioxin favored T_{reg} cell differentiation and suppressed EAE, another toxin, 6-formylindolo[3,2-b]carbazole, retarded T_{reg} cell development, favored Th17 cell differentiation, and caused more EAE pathology (97).

These initial reports leave a number of questions unanswered regarding how different AhR ligands lead to markedly different outcomes. However, they are intriguing in that they provide a possible link between the environment and an autoimmune diathesis. Furthermore, they provide another potential therapeutic target for small molecules inhibitors aimed at biasing CD4$^+$ T cell differentiation toward T_{reg} cells and away from the Th17 cell pathway.

Conclusion

Current understanding of the role of iT_{reg} cells in the maintenance of immune tolerance can best be described as a work in progress. Available evidence suggests that their antiinflammatory properties may be most critical at mucosal surfaces, where exposure to environmental microbial antigens, dietary factors and environmental products is most prominent. From a translational perspective, iT_{reg} cells differ from nT_{reg} cells in that polyclonal generation and expansion of the former can be accomplished easily *in vitro*, a potentially very useful property for immunotherapeutic applications.

Until its essential role in Th17 cell biology was discovered, TGFβ1 was believed to possess primarily antiinflammatory, antimitotic, and profibrotic properties. The shared requirement for this cytokine in iT_{reg} and Th17 cell differentiation leads naturally to the hypothesis that an imbalance between these two cell types may lead to tissue inflammation, primarily at but not necessarily restricted to mucosal surfaces (Fig. 1). By extension, this hypothesis offers numerous potential pharmacologic targets for immunomodulation. The rapid pace of basic science advances in this field paired with animal models of human diseases offers numerous opportunities for the practical application of this hypothesis in the near future.

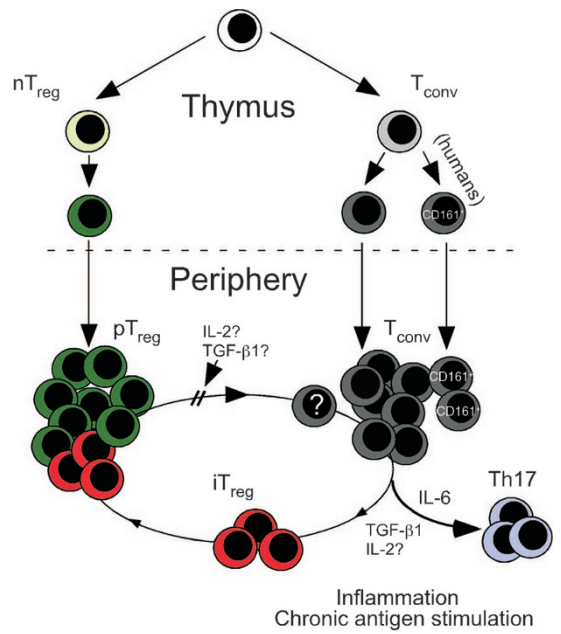

Figure 1. Induced T_{reg} (iT_{reg}) cells and Th17 cells are derived from a common naïve conventional T (T_{conv}) precursor population in mice. In humans, Th17 cells may come from a unique CD161$^+$ subset derived in the thymus. Both iT_{reg} and Th17 cells require TGFβ1 for their development, although the Th17 pathway is favored in the presence of IL-6. The peripheral T_{reg} (pT_{reg}) cell pool is comprised of iT_{reg} cells and "natural" T_{reg} (nT_{reg}) cells derived in the thymus. Both IL-2 and TGFβ-1 may stabilize Foxp3 expression, although some T_{reg} cells can ultimately lose it. The fate of cells that exit the pT_{reg} compartment is unknown, although recent data suggest that they could survive and develop into another type of Th cell.

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