

## Suppressor T Cells in Human Diseases

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Although central and peripheral tolerance are important for the regulation of human immune responses to self- and microbial antigens, an important role of suppressor CD4<sup>+</sup>CD25<sup>+</sup> T cells is suggested from the recent investigations of human autoimmune diseases and HIV. These new data provide increasing evidence that altered function of CD4<sup>+</sup>CD25<sup>+</sup> T cells may be an important factor in a wide range of human inflammatory and infectious diseases.

A curious finding in our understanding of human autoimmune diseases has been the presence of self-reactive T cells in the circulation of healthy individuals, as measured by in vitro cloning techniques, without evidence of disease (1). Although central tolerance clearly exerts a major effect in selecting the repertoire of T cells recognizing self-antigens, it is likely that peripheral tolerance is not the sole protector against autoimmune disease. Moreover, although the field of immunology discarded suppressor T cells almost two decades ago, when prevailing views of their mechanism could not stand the scrutiny of molecular biology, it was clear to investigators performing experiments in animal models of autoimmune disease that populations of T cells existed that could be adoptively transferred to prevent the onset of clinical disease.

The past decade has seen the discovery of several T cell populations that appear to have major regulatory effects on T cells responding to both self-antigens and those derived from infectious agents. As is frequently the case, these regulatory T cell populations were discovered first in experimental animal models and subsequently identified in humans. They can broadly be divided into T cells that appear to require antigen-specific, MHC-restricted stimulation, with subsequent secretion of cytokines that down-regulate immune responses—the prototypic Tr1 and Th3 cells, which secrete IL-10 and TGF- $\beta$ , respectively (2–5)—or more innate regulatory cells that do not appear to require an in vivo “adaptive” immunization to observe their function—the CD1-restricted NKT cells with invariant TCRs, “nonclassical” NK T cells with variant TCRs (6, 7), and the CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (8, 9).

After a decade of investigation in animal models of human disease, a series of recent articles in the *Journal of Experimental Medicine*, including this issue, have placed CD4<sup>+</sup>CD25<sup>+</sup> T cells as regulators of human immune responses in both autoimmune (10–12) and infectious diseases (13). The potential role of CD4<sup>+</sup>CD25<sup>+</sup> T cells in human disease presents a striking example of the essential interaction between basic investigation in animal models, both in vivo and in vitro, and clinical investigation of human disease, allowing a greater degree of confidence in the validity of the in vitro observation in humans.

*From Mouse Regulatory T Cells to Human.* The discovery of CD4<sup>+</sup>CD25<sup>+</sup> T cells was based on a simple in vivo observation: mouse thymectomy on neonatal day 3 leads to the development of multiorgan autoimmune disease (14). Seminal experiments performed by Sakaguchi and coworkers demonstrated that it was the depletion of CD4<sup>+</sup>CD25<sup>+</sup> cells that resulted in the onset of systemic autoimmune diseases in these neonatally thymectomized mice (15, 16), and cotransfer of these cells with CD4<sup>+</sup>CD25<sup>-</sup> cells prevented the development of experimentally induced autoimmune diseases such as colitis, gastritis, insulin-dependent autoimmune diabetes, and thyroiditis (17–23). In sum, these investigations demonstrated that in mice CD4<sup>+</sup>CD25<sup>+</sup> cells can regulate the responses of autoreactive T cells in vivo. The discovery of the importance of the transcriptional regulator FoxP3 in mouse CD4<sup>+</sup>CD25<sup>+</sup> T regulatory cell function (24, 25) and the previous observations that patients with IPEX (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance), a severe inflammatory disease similar to that seen in mice deficient in CD4<sup>+</sup>CD25<sup>+</sup> regulatory cells, have mutations in *FOXP3* (26), provided a direct correlation between an autoimmune animal model, mouse regulatory T cells, and a human autoimmune disease.

*Defining Human CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cells.* However, then how could CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells be investigated in humans? The in vitro assay developed by Shevach and coworkers (27), where the addition of mouse CD4<sup>+</sup>CD25<sup>+</sup> T cells to CD4<sup>+</sup>CD25<sup>-</sup> T cells resulted in suppression of proliferation at ratios of up to ~1:10 suppressor to target T cells, provided a convenient assay of T regulatory function. However, if a human CD4<sup>+</sup> T cell population inhibits the proliferation of other T cells in vitro and was isolated solely by expression of CD25, is it

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the same CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cell population observed in mice where CD4<sup>+</sup>CD25<sup>+</sup> cells are isolated from animals kept in germ-free facilities with low levels of endogenous T cell activation? Since CD25 can also be expressed by activated T cells, how can we know that the human CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cell populations now reported by many different groups (9, 28–30) are capable of suppressing immune responses *in vivo*? Since there is no unique cell surface marker expressed by regulatory T cells, it is difficult to enumerate and analyze these cells.

Heterogeneity of the isolated T regulatory cell populations may explain the contradictory findings as to the mode of action of CD4<sup>+</sup>CD25<sup>+</sup> T regulatory cells. Human CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells have been varyingly reported to suppress by mechanisms that involve either IL-10, or TGF- $\beta$ , or CTLA4, or none of the above (9, 28, 29, 31–34). During our own initial investigations to determine if suppressive CD4<sup>+</sup>CD25<sup>+</sup> T cells existed in human peripheral blood, we demonstrated that the separation of CD4<sup>+</sup> T cells into CD25<sup>neg</sup>, CD25<sup>med</sup>, and CD25<sup>high</sup> subsets by FACS<sup>®</sup> sorting resulted in the segregation of an IL-10<sup>-</sup>, TGF- $\beta$ <sup>-</sup>, and CTLA4-independent but cell contact-dependent suppression only with the CD25<sup>high</sup> subset (9). Moreover, the CD25<sup>high</sup> subset was predominantly CD62L<sup>high</sup> (>95%), a hallmark for resting cells that have not been recently activated. In contrast, the CD25<sup>med</sup> population exhibits functional variability *in vitro*: they can either enhance or suppress proliferation in cocultures and they actually contain the majority of recently activated CD62L<sup>lo/-</sup> CD4<sup>+</sup> cells in the circulation. Similar to mouse CD4<sup>+</sup>CD25<sup>+</sup> cells, these human CD4<sup>+</sup>CD25<sup>high</sup> T cells are unresponsive to *in vitro* antigenic stimulation (anergic) and strongly suppress the proliferation of responder T cells upon coculture (9).

*CD4<sup>+</sup>CD25<sup>+</sup> T Regulatory Cells in Human Autoimmune Disease.* The discovery of methods to evaluate the *in vitro* function of CD4<sup>+</sup>CD25<sup>+</sup> T cells in humans has allowed the importance of these regulatory T cells in human immune responses and disease to be examined. As discussed earlier, self-antigen-reactive T cells are present in healthy individuals and in patients with autoimmune disorders. The finding that autoreactive T cells found in patients with autoimmune disease are more easily activated than those from normal subjects (35, 36) leads to the hypothesis that regulatory T cells may normally suppress the activation of self-reactive T cells, and either deficient generation or reduced effector function of these CD4<sup>+</sup>CD25<sup>high</sup> cells plays a role in the development of autoimmunity. Consistent with this hypothesis is the recent work by Danke et al. demonstrating that deletion of CD4<sup>+</sup>CD25<sup>+</sup> T cells allows marked clonal expansion of human autoreactive T cells *in vitro* (37).

Multiple sclerosis (MS) is the most common neurologic disease of young adults (38). We compared the frequency and function of CD4<sup>+</sup>CD25<sup>high</sup> T cells derived from patients with MS with those from healthy control subjects. Although we found no change in the frequency of CD4<sup>+</sup>CD25<sup>high</sup> cells, there was a marked decrease in their effector function (10). Using CD4<sup>+</sup>CD25<sup>high</sup> populations

that were >98% CD62L<sup>high</sup>, the coculture suppression by patient-derived CD4<sup>+</sup>CD25<sup>high</sup> cells was 20%, whereas the suppression by CD4<sup>+</sup>CD25<sup>high</sup> cells isolated from healthy control subjects was 80%. Differences were also apparent in single cell cloning experiments, in which the cloning frequency of CD4<sup>+</sup>CD25<sup>high</sup> T cells was substantially reduced in patients compared with normal controls. As the strength of signal delivered through the TCR of target T cells is one factor determining whether regulatory CD4<sup>+</sup>CD25<sup>high</sup> T cells can suppress the responder T cell proliferation (39), several different strength stimuli were used to properly examine the function of CD4<sup>+</sup>CD25<sup>high</sup> T cells in controls and in patients with MS. A strong signal provided by maximal concentration of plate-bound anti-CD3 mAb abrogated suppression in both patient and control cocultures. In contrast, using lower concentrations of plate-bound anti-CD3 allowed a defect in the suppressive ability of CD4<sup>+</sup>CD25<sup>high</sup> regulatory cells derived from patients with MS to become apparent. Importantly, comixing experiments demonstrated that the decrease in T cell regulatory function was due to a defect in the CD4<sup>+</sup>CD25<sup>high</sup> T cell subset rather than the possibility that the responder CD4<sup>+</sup>CD25<sup>-</sup> T cells were refractory to suppression because of the presence of activated autoreactive T cells in the patient samples.

The potential involvement of CD4<sup>+</sup>CD25<sup>high</sup> T cells in human autoimmune polyglandular syndromes (APS), in which several organ-specific autoimmune diseases are clustered, has also been investigated recently (11). There are two variants of APS: type I, which is caused by loss of central tolerance, and type II (APS-II), which is of unknown etiology. As depletion of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in mice causes a syndrome with multiple endocrinopathies resembling APS-II, Kriegel et al. (11) examined whether defects in peripheral CD4<sup>+</sup>CD25<sup>high</sup> T cells would be found in APS-II. As observed in MS, CD4<sup>+</sup>CD25<sup>high</sup> T cells in patients with APS-II were found at normal frequency but were defective in their suppressive capacity. Furthermore, as in patients with MS the defect was shown not to be due to responder cell resistance, suggesting that the pathogenesis of APS-II is related to the function of these regulatory T cells.

Alterations in regulatory CD4<sup>+</sup>CD25<sup>high</sup> T cells have also been reported in patients with rheumatoid arthritis (40) and in patients with juvenile idiopathic arthritis (41). These studies examined CD4<sup>+</sup>CD25<sup>+</sup> cells directly at the sites of inflammation and in the peripheral blood, although the potential involvement of IL-10 or TGF- $\beta$  were not examined. Interestingly, the frequency of CD4<sup>+</sup>CD25<sup>high</sup> T cells was much greater in the synovial fluid compared with the peripheral blood in adult patients with rheumatoid arthritis. Patients with the juvenile disease also exhibited similar increases in synovial fluid CD4<sup>+</sup>CD25<sup>high</sup> cells; however the patients with the more favorable prognostic form of the disease also exhibited an increased frequency of CD4<sup>+</sup>CD25<sup>high</sup> T cells in the peripheral blood. When these regulatory T cells were isolated from the joint and tested *in vitro*, they demonstrated suppressive activity. As these studies find differences in the frequency of CD4<sup>+</sup>CD25<sup>high</sup> regulatory T cells isolated from the target tissue compared with the peripheral blood, they

suggest that regulatory T cells migrate into the joint and are either inactivated by inflammatory mediators, or alternatively, the enhanced number of regulatory T cells in the synovium may actually initiate the resolution of the episode of immune activity. The observation that the form of JIA with better prognosis correlates with enhanced numbers of peripheral CD4<sup>+</sup>CD25<sup>high</sup> cells is also of interest since it suggests that peripheral expansion of regulatory T cells may occur in this subset of patients. However, the major question remains as to whether regulatory T cells are able to function in the synovial fluid milieu as rheumatologic disease exists in the target tissue in the face of an enhanced number of regulatory T cells that are highly suppressive *in vitro*.

A new study by Ehrenstein et al. published in this issue (12) further suggests that regulatory T cells may be expanded *in vivo* in patients with rheumatoid arthritis that respond favorably to treatment with anti-TNF $\alpha$  therapy. Specifically, they demonstrate that the CD4<sup>+</sup>CD25<sup>high</sup> cells isolated from the peripheral blood of patients with active rheumatoid arthritis can suppress the responder CD4 T cell proliferative response but not the secretion of inflammatory cytokine. In contrast, CD4<sup>+</sup>CD25<sup>high</sup> cells isolated from patients after anti-TNF $\alpha$  therapy suppress both proliferation and cytokine secretion. These data may underscore the central role for cytokines in maintaining the inflammatory state *in vivo*. The authors demonstrated that the ability of bead-isolated CD4<sup>+</sup>CD25<sup>+</sup> T cells to induce a "suppressive phenotype" after culture with CD4 cells did not occur with regulatory T cells isolated from patients with active disease but did occur in cells isolated after anti-TNF $\alpha$  therapy. Moreover, the suppressive activity was restricted to the CD25<sup>high</sup> subset compared with CD4<sup>+</sup>CD25<sup>lo</sup> cells. Although this analysis focused on cells in the blood compared with the synovium, the correlation between anti-TNF $\alpha$  responders, CD4<sup>+</sup>CD25<sup>high</sup> frequency, and *in vitro* suppressor activity suggests that the activity of regulatory T cells in the peripheral blood may reflect what is occurring in the affected joints.

*A Role for CD4<sup>+</sup>CD25<sup>+</sup> T Regulatory Cells in HIV.* Also in this issue, the importance of CD4<sup>+</sup>CD25<sup>high</sup> regulatory T cells is extended with a report examining a role for regulatory cells in HIV infection. HIV is associated with loss of CD4<sup>+</sup> T cells and progressive CD4<sup>+</sup> T cell immune dysfunction, leading to impaired HIV responses early after infection (13). Kinter et al. hypothesized that the basis for the decrease in response to HIV relates to defects in CD25<sup>+</sup>CD4<sup>+</sup> regulatory T cells. In fact, they observed that in the majority of healthy yet HIV-infected individuals, CD25<sup>+</sup>CD4<sup>high</sup> T cells significantly suppressed cellular proliferation and cytokine production by CD4<sup>+</sup> and CD8<sup>+</sup> T cells in response to HIV antigens/peptides *in vitro*. As in patients with MS, these *in vitro* effects were cell contact dependent and IL-10 and TGF- $\beta$  independent. Paradoxically, they also found that patients with strong HIV-specific CD4<sup>+</sup>CD25<sup>high</sup> function *in vitro* had significantly lower levels of plasma viremia and higher CD4<sup>+</sup> to CD8<sup>+</sup> T cell ratios than patients without regulatory T cell activity. These data suggest that suppression of CD4 T cell activation by regulatory T cells may make HIV replication less favorable,

possibly because these cells suppress the activation of CD4<sup>+</sup> T cells and thus make fewer target cells available.

*Concluding Remarks.* It appears that CD4<sup>+</sup>CD25<sup>high</sup> regulatory cells modulate immune responses to self-antigens and infectious agents in humans. The possibility that CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells may have expanded *in vivo*, one interpretation of the observed increases in regulatory T cell frequency in the peripheral blood of patients with JIA and rheumatoid arthritis, indicates that the design of therapies that induce CD4<sup>+</sup>CD25<sup>high</sup> cell expansion may be of clinical relevance. The parallel observations relating CD4<sup>+</sup>CD25<sup>+</sup> regulatory cells in animal models of autoimmunity and human inflammatory diseases indicate that suppressor cells are back to stay as central regulators of immune responses in human diseases.

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