Costimulatory signals controlling regulatory T cells

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The concept of specialized suppressor T cells (now commonly called regulatory T cells or Tregs), which was abandoned by most immunologists in the early 1980s (1), was rescued by the description of a marker for their identification and isolation by Sakaguchi *et al*. (2), who, in 1995, described a $CD4^+CD25^+$ subpopulation of T cells that could prevent pathology in a mouse autoimmune disease model. However, the marker CD25, the α-chain of the high-affinity IL-2 receptor, is also induced on conventional T cells after activation. The dilemma arose as to how one could distinguish genuine Tregs from recently activated conventional T cells. Furthermore, the suppressor activity of $CD4^+CD25^+$ Tregs *in vitro* depended on their prior stimulation and could be overcome by strong costimulation (3, 4). What were the signals that controlled the activity of $CD4^+CD25^+$ Tregs *in vivo* that allowed them to inhibit certain immune responses but not others?

Glucocorticoid-Induced Tumor Necrosis Factor Receptor (GITR) and GITR Ligand (GITRL)

In a recent issue of PNAS, Tone *et al.* (5) reported the cloning of the murine ligand for GITR, a tumor necrosis factor receptor superfamily member that had previously been demonstrated to regulate $CD4^+CD25^+$ Treg function. Searching for novel Treg markers, two groups had independently found that freshly isolated $CD4^+CD25^+$ Treg cells but not conventional $CD4^+CD25^-$ T cells expressed uniformly high levels of GITR (6, 7). Activation of the $CD4^+CD25^-$ T cells *in vitro* rapidly increased GITR surface expression to levels comparable to that on $CD4^+CD25^+$ Tregs. GITR thus was no better than CD25 in distinguishing Tregs from activated conventional T cells. However, the addition of anti-GITR antibodies to the coculture of $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells *in vitro* produced a dramatic functional effect, completely abrogating suppression (6, 7). This effect appeared to be caused by active signaling into the $CD4^+CD25^+$ Tregs rather than blockade of interaction with the unknown putative GITR ligand. *In vivo*, the administration of anti-GITR antibody to young mice caused autoimmune gastritis, a disease also seen with depletion of $CD4^+CD25^+$ Tregs (6). Although these studies established GITR as a receptor on the surface of $CD4^+CD25^+$ Tregs that could modulate Treg function, the physiological role of GITR signaling remained elusive. Tone *et al.* (5) identified the murine ligand for GITR (GITRL) and provided some initial insight into the regulation of its expression. They showed that GITRL was expressed by antigen-presenting cells (APCs), i.e., dendritic cells, macrophages, and B cells, but not by T cells. Stimulation of dendritic cells with lipopolysaccharide *in vitro* led to a transient up-regulation of GITRL expression. Furthermore, GITR ligation by its ligand not only affected Tregs, but GITR signaling additionally had a costimulatory effect on purified conventional $CD4^+CD25^-$ T cells.

The article by Tone *et al.* is an important step toward understanding the regulation of Treg function *in vivo*, but more work is clearly needed. It will be interesting to look in more detail at GITRL expression patterns on different types of APCs including subtypes of dendritic cells *in vivo*, the relationship between the expression of GITRL and other costimulatory ligands, and the kinetics of GITRL expression during immune responses *in vivo*. It is tempting to speculate on the phenotype of GITR or GITRL transgenic or knockout mice. Will overexpression of GITRL lead to a breakdown of peripheral tolerance by "switching off" $CD4^+CD25^+$ Treg cells? Could dysregulated expression of GITRL thus be a contributing factor in the development of autoimmune diseases? Another important aspect of the article by Tone *et al.* is the formal demonstration that GITR ligation has a costimulatory effect on conventional $CD4^+$ T cells. This finding corrects the impression evoked by the two original papers describing GITR expression on $CD4^+CD25^+$ Tregs, that GITR signaling was unique to $CD4^+CD25^+$ Tregs. GITR ligation rather functions as a second signal for both $CD4^+CD25^+$ Tregs and conventional $CD4^+CD25^-$ T cells. Importantly, freshly isolated $CD4^+CD25^-$ T cells seem to be $GITR^{low}$ rather than $GITR^-$ much in contrast to $CD4^+CD8^+$ double positive thymocytes that appear to be truly $GITR^-$ (6).

The Two-Signal Model of T Cell Activation Revisited

A major paradigm of current immunology is that the initiation of an immune response requires an antigen-specific first signal and additional second signal(s) induced by "danger" or "foreignness." A popular version of the two-signal model for T cell activation, described a few years ago, focused on B7-1 and B7-2 molecules expressed on APCs as the main providers of T cell costimulation (8). In this model, T cells are activated through the interaction of peptide/MHC complexes and B7-1/B7-2 molecules on the APC with, respectively, the T cell antigen receptor (first signal) and constitutively expressed CD28 (second signal). Full T cell activation induces expression of the negative regulatory molecule CTLA-4, an alternative ligand for B7-1 and B7-2, which then turns off the immune response. Over the past several years, it has become clear that the initiation of an immune response is a much more complex process. Additional B7 family members expressed on APCs and their respective ligands on T cells have been identified, including B7h/ICOS and the negative regulatory PDL1+2/PD-1 (9). Furthermore, other classes of receptors have been shown to be involved in the regulation of immune responses, including tumor necrosis factor receptor family members like OX40, 4-1BB, and their ligands (10). Some of these costimulatory ligand/receptor pairs are constitutively expressed, whereas others are induced upon activation. There appears to be a hierarchy of costimulatory ligand/receptor combinations in the control of immune responses, e.g., CTLA-4 knockout mice die within 3–4 weeks from a severe lymphoproliferative syndrome (11), whereas PD-1 knockout mice develop a far more subtle lupus-like disease later in life (12). In fact, costimulatory receptors appear to differentially affect certain aspects of the immune response such as proliferation, survival, and effector cell differentiation (10). Another layer of complexity is added to the two-signal model by the demonstration of the $CD4^+CD25^+$ Treg cells. The general implication is that the initiation of a T cell response involves the interaction of three different cell types (APCs, conventional T cells, and regulatory T

See companion article on page 15059 in issue 25 of volume 100.

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cells, $CD4^+CD25^+$ Tregs being one example). Costimulatory signals provided by APCs influence the "behavior" of both the conventional T cells and the regulatory T cells. The interaction of the three (or more?) cell types will then "decide" about the initiation and phenotype of the response. An example of this complex interplay is the recent demonstration that conventional T cells need a certain cytokine milieu, with an absolute requirement for IL-6 produced by dendritic cells, to escape from the suppressive influence of the $CD4^+CD25^+$ Treg cells (13).

Costimulatory Molecules on $CD4^+CD25^+$ Treg Cells

Currently, no surface molecules are known that are truly specific for $CD4^+CD25^+$ Tregs, GITR being no exception. This means that Treg activity is regulated by the same ligands that regulate conventional T cells. What is known about costimulatory molecules and their functional effect on $CD4^+CD25^+$ Tregs? As discussed above, GITR ligation on $CD4^+CD25^+$ Tregs appears to abrogate their "suppressiveness." How this works is not clear, in keeping with the fact that the suppressor mechanism of $CD4^+CD25^+$ T cells still awaits elucidation. The classical costimulatory molecule CD28 is expressed on the surface of $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells at equivalent levels. As for anti-GITR, addition of anti-CD28 antibodies abrogates suppression in coculture systems *in vitro* (4); however, this appears to be caused by a costimulatory effect on the $CD4^+CD25^-$ T cells rather than inhibition of the Tregs (14). Interestingly, CD28 knockout mice and B7-1/B7-2 double knockout mice have a much reduced frequency of $CD4^+CD25^+$ Treg cells. On the autoimmune genetic background of the NOD mouse, this regulation correlates with earlier disease onset (15). Thus, an additional function of B7/CD28 interaction seems to be the regulation of the $CD4^+CD25^+$ Treg pool size (16). This finding is probably an indirect effect through the control of IL-2 production by conventional T cells, given that $CD4^+CD25^+$ T cells are incapable of producing IL-2 themselves but require IL-2 signals for their generation and/or homeostasis (17). CTLA-4, much like GITR, is highly expressed in freshly isolated $CD4^+CD25^+$ T cells although it predominantly resides intracellularly (18). It has been proposed that CTLA-4 signaling switches off $CD4^+CD25^+$ Tregs; however, this view is not universally accepted. CTLA-4 is up-regulated in $CD4^+CD25^-$ T cells upon activation. More recent concepts of CTLA-4 function suggest that it inhibits the expansion of high-affinity T cell clones within a polyclonal responder population (19). The role of CTLA-4 for $CD4^+CD25^+$ Treg cells could thus be to maintain a broad range of T cell antigen receptor specificities and, assuming a largely self-restricted repertoire, prevent the development of repertoire holes that could result in autoimmune responses. Finally, a number of other tumor necrosis factor receptor family members appear to be differentially expressed on $CD4^+CD25^+$ Tregs (20). However, no evidence has been reported yet that any of these can be used as additional Treg markers, or that they have functions similar to GITR. Signaling via GITR is unique in that it both turns off $CD4^+CD25^+$ Tregs and directly costimulates conventional T cells. The reported cloning of the murine ligand for GITR and initial characterization of GITRL expression is another step forward in understanding the regulation of Tregs and the complexities involved in the induction of immune responses.

GITR both turns off regulatory T cells and costimulates conventional T cells.

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