

causes a bend in the nucleic acid backbone (Fig. 1). Proteins containing HMG boxes that regulate genes specific to T cells have been described before and include T cell factor (TCF), lymphoid enhancer factor (Lef) and HBP1 (HMG box-containing protein 1)<sup>9–11</sup>. As the special architectural arrangement of DNA and associated proteins is thought to be important in the regulation of gene expression, a protein that can impose structural changes, such as bending, would be a major player in this process (Fig. 1). This property and the pattern of TOX expression (which is highest in the thymus) prompted the authors to examine its role in thymocyte differentiation<sup>1</sup>.

Apart from the strangely high amounts of TOX expressed at the very early stages of thymocyte development (DN1 and DN2) its expression pattern suggested an involvement in the DP to SP transition phase<sup>1</sup>. From here on its role becomes less clear, as mature naïve cells responding to antigen no longer modulate its expression<sup>1</sup>.

More striking, however, are the results from transgenic mice that express TOX. Although such mice do not show overt changes in their overall thymus cellularity, they show an increase in the production of CD8<sup>+</sup> SP thymocytes at the expense of SP CD4<sup>+</sup> T cell numbers. These CD8<sup>+</sup> SP T cells also appear to express TCRs that, under normal circumstances, are preferentially expressed on CD4<sup>+</sup> SP T cells. What is even more intriguing is the fact that the appearance of these cells is unaffected by the absence of MHC class I. One is tempted to argue that TOX over-expression has removed the need for CD4 coreceptor signaling, so that even those cells that would normally be lost because of CD4 down-regulation are now res-

cued. Nevertheless, in the absence of evidence that such cells may have been selected on MHC class II it is too early to draw firm conclusions.

Kaye and colleagues propose that TOX affects lineage commitment and, when over-expressed, leads to the CD8 pathway of differentiation, regardless of TCR specificity<sup>1</sup>. There is no question that, in this case, genetic manipulation has caused a distortion in lineage commitment<sup>1</sup>; similar perturbations in lineage commitment have been reported in other cases, such as with CSK (COOH-terminal Src kinase)-deficient mice<sup>12</sup>. In the article by Kaye and colleagues<sup>1</sup>, genetic manipulation disrupted TOX expression both in quantitative terms (over 20 times more protein was found in the thymus of TOX-transgenic compared to wild-type mice) and in qualitative terms (the fine developmental regulation of TOX expression during the DN1→DN4 and DN4→DP→SP transitions was lost) so that uniformly high expression occurred throughout development. In addition, because high TOX expression was maintained continuously, it is likely that downstream targets were also activated or silenced, which induced the phenotypic repercussions observed<sup>1</sup>.

The article by Kaye and colleagues<sup>1</sup> opens an exciting new avenue within the field of thymocyte differentiation and future work should clarify the precise points at which TOX regulates thymocyte development. My prediction is that, in most cases, no one single gene will determine the lineage commitment of a cell. It is unlikely that, during evolution, nature would have put all its eggs in one basket. Witness the redundancy identified in gene knock-out studies or the presence of multiple members of a gene family that can

“stand-in” when a relative is absent. A more plausible scenario is the careful orchestration of the expression of molecules that turn the cell in one direction or another. All these molecules probably contribute, to a certain extent, to lineage commitment, and changes in expression, amounts or timing are likely to ensure that the precise events take place at the correct time. Undoubtedly, disrupting the expression of any one of these molecules with the use of transgenes or knock-out deletions is bound to yield important and valuable information, such as that reported in the article by Kaye and colleagues<sup>1</sup>. We should always, however, remain open to potentially different interpretations. Only the gradual accumulation of information, the correlation between the different findings and constructive discussion of new data will lead to future breakthroughs in the understanding of how thymocyte development and lineage commitment works.

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## T<sub>S</sub> cells and immune tolerance induction: a regulatory renaissance?

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Within the complex biology of immune system regulation, mechanisms that down-modulate host immune responses are likely to be equally important as those that activate them. Proper regulation of immune responses requires the effective and timely action of mechanisms that shut-off host immune

responses. In this way, uncontrolled lymphocyte proliferation upon exposure to antigenic stimuli can be avoided and the generation of aberrant immune responses that target host antigens and lead to the development of autoimmune diseases can be prevented. The concept of suppressor T (T<sub>S</sub>) cells was first

CD8<sup>+</sup> T<sub>S</sub> cells induce antigen-specific tolerance. The T<sub>S</sub> cells may do this by increasing the expression of inhibitory receptor ILT3 and ILT4 on DCs, rendering these cells tolerogenic to CD4 cells.

developed in the 1970s; it was envisioned that this subset of lymphocytes was responsible for the active control, and ultimately the termination, of immune responses<sup>1</sup>. T<sub>S</sub> cells became disfavored in the 1980s and early 1990s, mainly because of difficulties in identifying a distinct phenotype for antigen-specific T<sub>S</sub> cells.

As the popularity of  $T_S$  cells waned, studies on how immunological tolerance is achieved focused upon the central and peripheral mechanisms of clonal deletion. However,  $T_S$  cells have re-emerged as a subject of intense research because of their potential role in eliciting immunological tolerance. In this issue of *Nature Immunology*, Chang *et al.*<sup>2</sup> explore the immunomodulatory effects of a  $CD8^+ T_S$  lymphocyte population that appears to modulate the function of antigen-presenting cells (APCs), potentially rendering them tolerogenic in a transplant setting.

Transplantation immunology research has long focused on the development of methods with which to induce specific immunological “tolerance” to transplanted organs (allografts) without the need for life-long treatment with nonspecific and often toxic immunosuppressive drugs. The quest for therapies that might induce specific immune tolerance—ideally *via* short-term interventions that would target only the pathogenic immune responses and leave protective host immune responses unimpaired—has provided a “holy grail” for transplant immunologists. If attainable, such tolerogenic interventions would also have great potential for the treatment of autoimmune diseases. It is within this context that renewed attention has been focused on the  $T_S$  cells.

Several subsets of  $T_S$  and T regulatory ( $T_R$ ) cells, with distinctive phenotypes and proposed mechanisms of, have now been identified

(**Fig. 1**). These include  $CD4^+CD25^+$  T cells that express cytolytic T lymphocyte-associated antigen 4 (CTLA-4); a subset of  $CD4^+$  T cells, defined as T helper 3 ( $T_{H3}$ ) and/or  $T_{R1}$  cells (and which may also have a  $CD8^+$  phenotype); and a subset of  $CD8^+CD28^-$  T cells that suppress alloreactive immune responses in an antigen-specific, major histocompatibility complex (MHC)-restricted manner<sup>3–6</sup>. The regulatory effect of the  $CD4^+CD25^+$  subset of  $T_R$  cells is thought to be cytokine-independent but appears to require direct T cell–T cell contact and, most likely, expression of the costimulatory molecule CTLA-4<sup>3,4,7</sup>. In contrast, the  $T_{H3}$  or  $T_{R1}$  cells inhibit normal  $CD4^+$   $T_H$  cell reactivity *via* the production and release of the immunomodulatory cytokines transforming growth factor- $\beta$  (TGF- $\beta$ ) and interleukin-10 (IL-10), respectively<sup>4,5</sup>. In murine systems, both  $CD4^+CD25^+$  T cells and  $T_{H3}$  or  $T_{R1}$  cells can mediate a “suppressor-regulatory” effect on alloreactive T cells<sup>3–5</sup>. Importantly, both subsets of  $CD4^+$   $T_R$  cells act directly on other  $CD4^+$   $T_H$  cells.

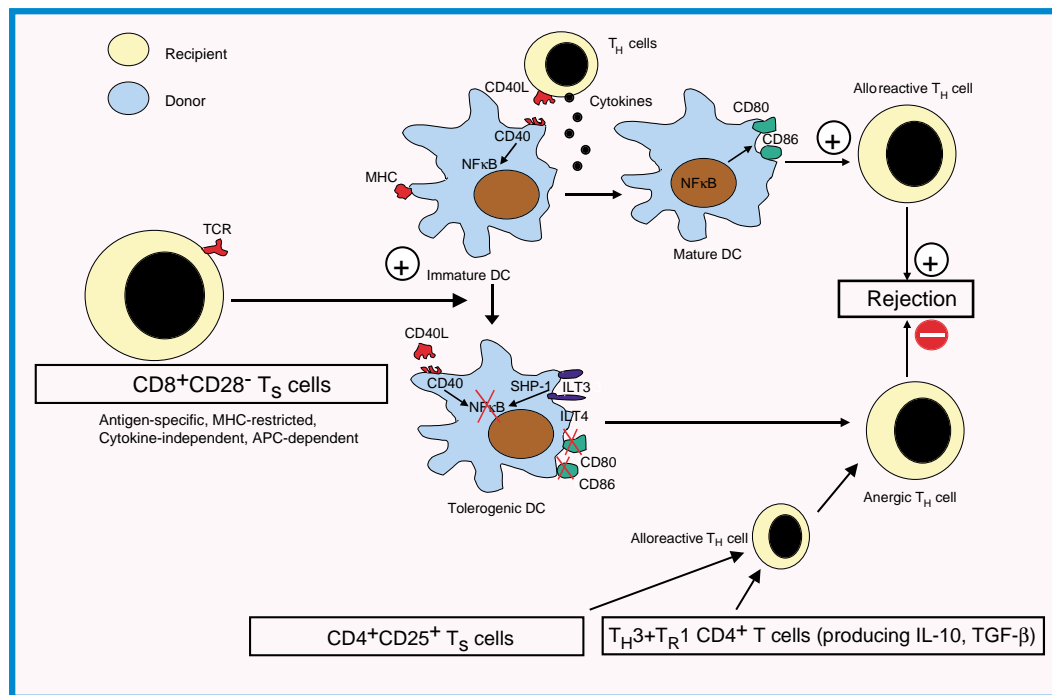
Chang *et al.*<sup>2</sup> generated  $CD8^+CD28^-$   $T_S$  cells *in vitro* after multiple rounds of stimulation with allogeneic or xenogeneic APCs. Exposure of immature dendritic cells (DCs) to such  $CD8^+CD28^-$   $T_S$  cells appears to result in interference with CD40–CD40 ligand (CD40L)-mediated signaling<sup>2</sup>; this signaling normally induces NF- $\kappa$ B activation and thereby prevents the functional maturation of DCs and their high expression of costimulatory

molecules (including CD80 and CD86)<sup>2</sup>. The tolerogenic influence of  $CD8^+CD28^-$   $T_S$  cells is associated with induced expression, on the DC surface, of the inhibitory molecules immunoglobulin-like transcript 3 (ILT3) and ILT4<sup>2</sup>. Both these molecules contain an inhibitory “ITIM” motif in their cytoplasmic domains and mediate inhibition of cell activation *via* recruitment of the modulatory tyrosine phosphatase SHP-1<sup>2</sup>. The resulting  $CD8^+CD28^-$   $T_S$ -induced  $CD80^+CD86^-$  ILT3<sup>+</sup>ILT4<sup>+</sup> DCs (of donor origin) were then able to anergize, in a HLA-restricted manner, alloreactive  $CD4^+$   $T_H$  cells (of recipient origin) in tissue culture<sup>2</sup>. Thus, in contrast to the postulated mechanisms of  $CD4^+$   $T_R$  cell action, it is proposed that the effect of the  $CD8^+CD28^-$  phenotype is facilitated by a cellular intermediary, the so-called tolerogenic DC<sup>2</sup>. Thus, a new pathway of cross-talk between different T cell subsets, which uses allogeneic DCs as a functional bridge, has been identified<sup>2</sup> (**Fig. 1**). Based on the tolerogenic influences of  $CD8^+CD28^-$   $T_S$  cells that Chang *et al.* observed *in vitro*, it is proposed that this type of  $T_S$  cell might also serve as an agent for the induction of tolerance to allografts *in vivo*<sup>2</sup>.

Although this model is provocative, the relevance of the  $CD8^+CD28^-$   $T_S$  cells generated in culture to those generated and operative *in vivo* must be clearly established. To what extent do the  $CD8^+CD28^-$   $T_S$  cells that Chang *et al.*<sup>2</sup> found in certain cardiac allograft recipients truly resemble those generated in tissue culture under artificial and potentially idiosyncratic

### Figure 1. Suppression of alloreactive immune responses by T cells.

It is proposed that recipient-derived  $CD8^+CD28^-$   $T_S$  cells interact with donor-derived DCs and, *via* functional blockade of CD40-mediated signaling and NF- $\kappa$ B activation, generate a tolerogenic DC phenotype (ILT3<sup>+</sup> or ILT4<sup>+</sup> and CD80<sup>+</sup>CD86<sup>-</sup>). Tolerogenic DCs, in turn, may interact with and anergize recipient-derived, alloreactive  $CD4^+$   $T_H$  cells. Other subsets of  $T_R$  cells that are reportedly involved in the suppression of alloimmune and autoimmune reactions include  $CD4^+CD25^+$   $T_R$  cells and  $T_{H3}$  or  $T_{R1}$  cells, which produce IL-10 and TGF- $\beta$ . These types of “suppressor”  $CD4^+$  T cells are believed to act directly upon alloreactive or autoreactive  $CD4^+$  T cells, resulting in their functional inactivation. In contrast, “suppressor”  $CD8^+CD28^-$  T cells are believed to act, *via* a DC intermediate, to induce antigen-specific anergy in responding  $CD4^+$  T cells.



circumstances? When and how might such CD8<sup>+</sup>CD28<sup>-</sup> T<sub>S</sub> cells be generated and exert their regulatory influence *in vivo*? Presumably, if this T<sub>S</sub> cell population actually contributes to more effective graft acceptance *in vivo*, it would need to act very soon after initial transplantation, as donor DCs are likely to persist in the recipient host for only a short period of time. Importantly, should the presence of such T<sub>S</sub> regulatory cells be verified in organ transplant recipients, it will be essential to investigate what role, if any, that such CD8<sup>+</sup>CD28<sup>-</sup> T<sub>S</sub> cells might also serve in the modulation of normal immune responses.

Chang *et al.* provide initial details of potentially key regulatory interactions<sup>2</sup>. However, important details concerning the interaction between T<sub>S</sub> cells and DCs and between DCs and T<sub>H</sub> cells await discovery. The events that lead to cross-talk between the ILT3 and/or ILT4→SHP-1 inhibitory signaling pathway(s) and the CD40-mediated activation of the NF-κB pathway need to be defined. In addition, it remains unclear whether the T cell receptor–MHC interaction results in the activation of specific signaling in DCs that result in ILT3 and ILT4 up-regulation, or whether a more complex set of interactions between T<sub>S</sub> cells and DCs are required to generate the tolerogenic phenotype. Although ILT4 binds various MHC class I molecules<sup>8</sup>, it is not clear how this interaction might be involved in the induction of CD4<sup>+</sup> T cell anergy. The ligand for ILT3 is currently unknown and, along with the nature of its effects on CD4<sup>+</sup> T cells, it will need to be identified. It will be similarly important to clarify whether ILT3 or ILT4 stimulation alone is sufficient to anergize CD4<sup>+</sup> T cells, or whether additional signals and pathways for CD4<sup>+</sup> T<sub>H</sub> cell modulation are required to obtain anergy. Although the results reported by Chang *et al.*<sup>2</sup> clearly indicate that the T<sub>S</sub> cell–tolerogenic DC system can inhibit alloreactive CD4<sup>+</sup> T cells in tissue culture, rejection of allografts is a far more complex (and incompletely understood) process that is not solely dependent on CD4<sup>+</sup> T cells<sup>9,10</sup>. It is also dependent on CD8<sup>+</sup> T, B and natural killer cells, all of which can act individually or in concert to promote allograft rejection. Likewise, given that the suppressor effects of

CD8<sup>+</sup>CD28<sup>-</sup> T<sub>S</sub> cells are reversible by exposure of anergic alloreactive CD4<sup>+</sup> T cells to IL-2, it is unclear how durable their beneficial activity might be *in vivo* in the face of an active rejection episode. Resolution of all these issues is essential if manipulations of this pathway of tolerance or suppression are to be successfully developed as therapeutic interventions for treating or preventing organ rejection in transplant recipients.

Translation of the results of basic studies of immune tolerance into effective therapies for the treatment of transplant rejection and autoimmune diseases represents a critical and complex challenge. The induction of tolerance to allografts is more difficult to achieve in human transplant recipients and preclinical experimental nonhuman primate models, such as rhesus macaques, than it is in experimental murine models. Recent exploration of new and theoretically selective immune interventions—such as the use of costimulatory blockade with monoclonal antibodies to CD40L and CTLA-4–immunoglobulin, depletion of specific T cell subsets and manipulation of the cytokine production or their end-organ effects—have been pursued in animal models of solid organ allografts<sup>11,12</sup>. Strategies for tolerance induction, such as the induction of a persistent state of chimerism (using nonmyeloablative bone marrow transplantation or donor lymphocyte transfusions) are also being actively investigated<sup>11</sup>. However, even highly complex protocols for tolerance-induction using multiple modalities of immunomodulatory interventions—such as those involving costimulatory blockade, donor cell transfusions and conventional immunosuppression—has to date, failed to prevent rejection in experimental nonhuman primate models when therapy is withdrawn (C. Larsen, personal communication).

Given that most studies of tolerance induction to allografts have been performed in murine model systems, the data from Chang *et al.*<sup>2</sup>—which support the role of ILT3 and ILT4 expression in generation of the “tolerogenic” function of DCs in a human tissue culture model of alloreactive immune response—are interesting. It has thus been hypothesized that ILT3 and ILT4 provide potential targets for

immunological interventions designed to either block (in immunodeficient patients) or trigger (in patients experiencing transplant rejection or with autoimmune diseases) their signaling pathway. From this perspective, the generation of small molecules or recombinant proteins that mimic the inhibitory effects of ILT3 and ILT4 stimulation on the generation of alloreactive (and potentially autoreactive) CD4<sup>+</sup> T cells will enable direct evaluation of the validity, relevance and relative importance of this regulatory pathway for tolerance induction, both in tissue culture models and *in vivo*. Should such reagents be developed and confirm the hypotheses of Chang *et al.*<sup>2</sup>, they might one day also provide new candidates for the beneficial therapeutic modulation of host immune responses in clinical transplantation and the treatment of autoimmune diseases.

The path to effective immunomodulatory strategies that enable tolerable and effective organ and tissue transplants, as well as those that effectively ameliorate autoimmune diseases, will likely continue to be long and challenging. At present, it remains difficult to gauge how close we are to assembling a coherent picture of which immunoregulatory pathways might be most effectively targeted, and how different aspects of the complex network of immune system control might be modulated in the most beneficial and synergistic ways. However, the perspective of the field understandably grows increasingly positive as new molecular pathways and targets for immune modulation are identified.

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