TGF-β puts the brakes on homeostatic proliferation

Charles D Surh & Jonathan Sprent

The cellular mechanism by which the cytokine TGF-β maintains the homeostasis of mature T cells and prevents the emergence of severe lethal lymphoproliferative disease has remained obscure. It is now shown that TGF-β restrains the homeostatic T cell proliferation driven by self ligands from erupting into overt autoimmunity.

Transforming growth factor-β (TGF-β) is a pleiotropic cytokine[1] whose precise effects depend on the cell lineage, the state of cell differentiation or activation, and the particular cytokine milieu. TGF-β exerts a considerable influence on the immune system and has an essential role in maintaining normal immune homeostasis. Thus, abrogation of TGF-β signaling, by deletion of either the cytokine or its receptor, leads to rapid and lethal lymphoproliferative disease[1]. The disease in these models is T cell driven and closely resembles the disorder that develops in mice deficient in Foxp3$^+$ regulatory T cells (T_{reg} cells)[2]. As TGF-β is essential for the development and function of T_{reg} cells, the onset of lymphoproliferation in TGF-β-deficient mice would seem to indicate that a defect in T_{reg} cells is the lynchpin of this disease. However, that apparently neat explanation does not fit. For example, the disease cannot be controlled by the adoptive transfer of T_{reg} cells into mice deficient in the receptor for TGF-β (TGF-βR)[3]. In this issue of *Nature Immunology*, Zhang and Bevan have now resolved the puzzle of why TGF-β deficiency causes lymphoproliferation[4].

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The authors generate a mouse model with conditional deletion of TGF-βRII on T cells at a late stage of T cell development through coexpression of a *lox*P-flanked gene encoding TGF-βRII (*Tgfbr2*$^{f/f}$) and Cre recombinase driven by the distal promoter of the gene encoding the kinase Lck (dLck-Cre). They find complete deletion of TGF-βRII on T cells in adult mice; however, the mice unexpectedly show no signs of lymphoproliferative disease and only a minor perturbance in normal T cell homeostasis. Those findings are in striking contrast to the considerable disease noted in previous studies of TGF-βR-deficient mice. Critically, in those studies, coexpression of *Tgfbr2*$^{f/f}$ and Cre driven by the promoter of the gene encoding the coreceptor CD4 (CD4-Cre) resulted in deletion of *Tgfbr2*$^{f/f}$ at a much earlier stage of thymic differentiation than its deletion in the TGF-βRII-deficient *Tgfbr2*$^{f/f}$ dLck-Cre mice studied by Zhang and Bevan[4].

After their release from the thymus, naive T cells generally remain in interphase for prolonged periods. When subjected to lymphopenic conditions, however, exposure to higher concentrations of cytokines, especially interleukin 7, causes resting T cells to proliferate slowly and gradually expand in number[5]. Such homeostatic proliferation occurs naturally in the neonatal period and also when adult T cells are transferred to lymphopenic hosts. With their *Tgfbr2*$^{f/f}$ dLck-Cre model, Zhang and Bevan assess the ability of TGF-βRII-deficient naive adult T cells to undergo lymphopenia-driven proliferation[4]. Notably, they find that instead of the typical slow homeostatic proliferation characteristic of wild-type T cells, the TGF-βRII-deficient naive T cells undergo massive proliferation reminiscent of an overt immune response. Moreover, the expanded T cell populations acquire prominent effector function, including expression of granzyme B and interferon-γ, and migrate in large numbers into liver, lung and intestine. Such conspicuous T cell activation occurs not only in hosts with chronic lymphopenia, such as RAG-1-deficient mice, but also in wild-type hosts made acutely lymphopenic by irradiation. This finding is of interest because the pattern of proliferation in hosts with acute lymphopenia is distinctly different from that in hosts with chronic lymphopenia[6]. With acute lymphopenia, proliferation is typically slow and driven by higher concentrations of cytokines plus contact of T cell antigen receptors (TCRs) with various ligands of self peptide and major histocompatibility complex (MHC). In contrast, a large component of the proliferation induced in hosts with chronic lymphopenia is directed against foreign environmental antigens and antigens derived from enteric commensal microbiota. As the considerable proliferation of TGF-βRII-deficient naive T cells is apparent during both types of lymphopenia, the surprising implication is that the proliferation is directed against self antigens rather than foreign antigens.

Two additional approaches provide direct evidence for the proposal of the involvement of self antigens as the target for proliferation. First, naive CD8$^+$ T cells from OT-I mice (which have transgenic expression of a TCR specific for chicken ovalbumin) on a TGF-βRII-deficient background undergo intense proliferation when transferred into syngeneic lymphopenic hosts, in contrast to the slow proliferation of wild-type OT-I cells. As the host mice do not express ovalbumin, the rapid proliferation of the TGF-βRII-deficient donor cells must be elicited by self antigens. The second approach involves measurement of the ability of TGF-βRII-deficient OT-I cells to respond to ovalbumin-derived altered peptide ligands (APLs) *in vitro*. Using a panel of APLs with a

range of affinity for OT-I cells, the authors find an inverse correlation between the TCR affinity of the peptide and its ability to induce greater population expansion of TGF-βRII-deficient OT-I cells than of wild-type (control) OT-I cells. Thus, the difference in the rate of proliferation of these two types of OT-I cells is minimal for high-affinity peptides and considerable for peptides of moderate affinity but much greater (~100-fold) for weak peptides. These findings are intriguing because weak peptides are known to provide the stimulus for initial positive selection of T cells in the thymus as well as the TCR signal needed for homeostatic proliferation. The objection that the data might reflect an artifact of TGF-βRII-deficient T cells is ruled out by the observation that the addition of neutralizing antibody to TGF-β markedly enhances the proliferation of wild-type OT-I cells in response to low-affinity peptide *in vitro*. Also, for high-affinity peptides, the *in vitro* data are confirmed *in vivo* by the finding that proliferative responses to ovalbumin expressed by infectious pathogens are much the same with TGF-βRII-deficient OT-I cells and wild-type (control) OT-I cells. Furthermore, the absence of TGF-βRII does not influence responses to cytokines, which indicates that the suppressive effect of TGF-β is restricted to TCR signaling.

The key conclusion from these experiments is that the suppressive influence of TGF-β applies selectively to weak TCR signaling, notably to naive T cells engaged in continuous interactions with self peptide–MHC ligands. This conclusion raises the question of why the lymphoproliferation in the Zhang and Bevan *Tgfbr2*$^{f/f}$ dLck-Cre model occurs only after adoptive transfer[4] yet developed spontaneously in previously published models of TGF-βRII deficiency. As suggested by the authors, the deletion of *Tgfbr2*$^{f/f}$ in their dLck-Cre model is induced relatively late in ontogeny and leads to residual expression of TGF-βRII by the cohort of new T cells released from the thymus in the neonatal period; uncontrolled lymphopenia-induced proliferation during this period is thus largely prevented by TGF-β signaling[4] (**Fig. 1**). In contrast, early deletion of TGF-βRII in the *Tgfbr2*$^{f/f}$ CD4-Cre model leads to unrestrained proliferation of newly exported TGF-βRII-deficient T cells in the neonatal period (**Fig. 1**).

The finding that the stimulus for proliferation in TGF-β-deficient mice is provided largely by self peptide–MHC ligands indicates that the severe pathology of these mice is a reflection of generalized autoimmune disease. Responses to foreign antigens seem to be unimportant, because TGF-β-deficient mice develop severe disease even when raised under germ-free conditions[7]. The data thus strengthen the view that TGF-β has a vital role in preventing

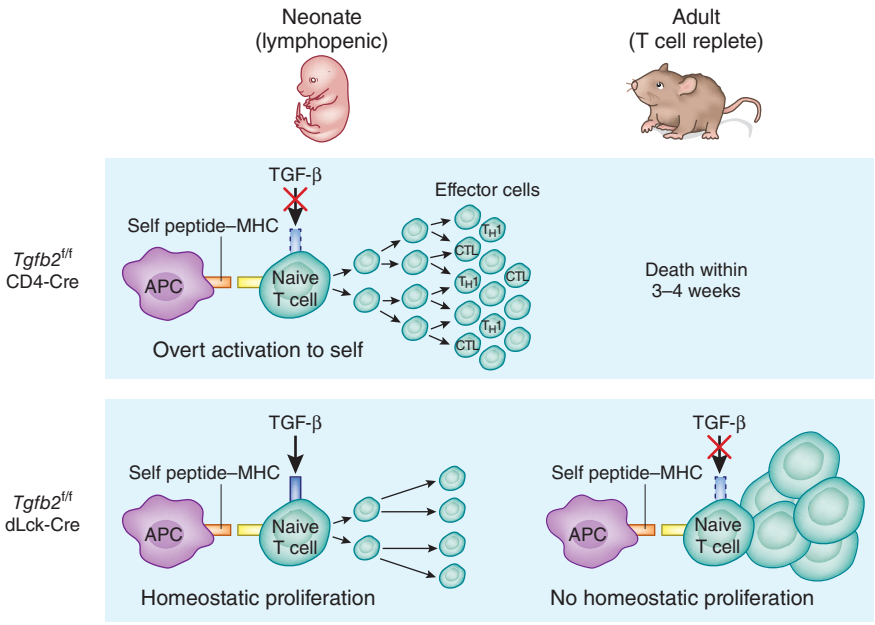

Figure 1 TGF-β signaling is essential in preventing homeostatic proliferation driven by self antigen from escalating into overt autoimmunity. Mice deficient in TGF-β or with *Tgfbr2*$^{f/f}$ alleles deleted by CD4-Cre during an early stage in thymic development (*Tgfbr2*$^{f/f}$ CD4-Cre; top) develop early-onset autoimmune disease that affects multiple organs and die within 3–4 weeks. In contrast, Zhang and Bevan now show that mice undergo deletion of *Tgfbr2*$^{f/f}$ via dLck-Cre (*Tgfbr2*$^{f/f}$ dLck-Cre; bottom) during a late stage in thymic development and have normal T cell homeostasis[4]. Expression of residual TGF-βRII on T cells from the late-deleting *Tgfbr2*$^{f/f}$ dLck-Cre mice during the neonatal period prevents unrestrained responses to self ligands. In these mice, complete deletion of TGF-βRII on T cells occurs only at the adult stage. However, because adults are not lymphopenic, the mice do not develop lymphoproliferative disease and remain healthy. APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte; T_H1, T helper type 1 cell.

autoimmune disease. The idea that suppression by TGF-β selectively limits responses to self ligands raises several questions. First, as T_{reg} cells have a decisive role in the avoidance of autoimmune disease in normal mice, why do they not prevent disease in TGF-β-deficient mice even after adoptive transfer of normal T_{reg} cells? One possibility is that T_{reg} cells are unable to suppress slow lymphopenia-driven proliferation in response to self ligands[8]. The impotence of normal T_{reg} cells in the TGF-β-deficient and *Tgfbr2*$^{f/f}$ CD4-Cre models might also reflect lower donor T_{reg} cell function caused by the abnormally high concentrations of TGF-β in the TGF-βR-deficient hosts. Alternatively, whereas wild-type T cells may be sensitive to TGF-β-mediated suppression, TGF-βR-deficient T cells are presumably resistant to such inhibition, although other suppressive mechanisms of T_{reg} cells would be expected to compensate for this deficiency. Clearly, further work is needed to resolve this issue.

Second, in published studies of mice with TGF-βRII deficiency driven by CD4-Cre and TGF-β-deficient mice[1,3,9,10], why did polyclonal T cells undergo considerable spontaneous proliferation but four independent TCR-transgenic lines (CD4$^+$ OT-II, TEα and DO11.10 and CD8$^+$ HY T cells) did not? In this context, it is notable that the ability of these four transgenic lines on a wild-type background to undergo homeostatic proliferation in lymphopenic hosts is minimal[5]. Hence, the intrinsic affinity of their TCR for self ligands may be very low. In contrast, the OT-I line studied by Zhang and Bevan undergo prominent homeostatic proliferation[4], suggestive of very high self-reactivity. Therefore, in future studies of the dLck-Cre model, it will be important to determine whether different results are obtained with other TCR-transgenic lines, for example, the CD8$^+$ HY line.

Third, in terms of responses to foreign antigens, why are strong TCR responses apparently resistant to suppression by TGF-β? This question is yet to be resolved; the simplistic answer is that strong responses are generally harder to inhibit than weak responses.

As a final comment, the appreciation that suppression by TGF-β is heavily influenced by TCR affinity adds an elegant extra layer of complexity to the mechanisms that shape self tolerance and self-nonself discrimination. For newly formed T cells, there is increasing evidence that a process of TCR 'tuning' restricts the reactivity of post-thymic T cells, allowing naive cells to receive TCR signals that are just sufficient to

ensure their survival but too weak to induce an overt anti-self response and, at the same time, unable to restrict strong responses to foreign antigen[11,12]. Through its selective inhibitory effect on weak TCR responses, TGF-β thus emerges as a key controller of TCR tuning.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Li, M.O. & Flavell, R.A. *Cell* **134**, 392–404 (2008).
2. Fontenot, J.D., Gavin, M.A. & Rudensky, A.Y. *Nat. Immunol.* **4**, 330–336 (2003).
3. Li, M.O., Sanjabi, S. & Flavell, R.A. *Immunity* **25**, 455–471 (2006).
4. Zhang, N. & Bevan, M.J. *Nat. Immunol.* **13**, 667–673 (2012).
5. Surh, C.D. & Sprent, J. *Immunity* **29**, 848–862 (2008).
6. Kieper, W.C. *et al. J. Immunol.* **174**, 3158–3163 (2005).
7. Boivin, G.P., Ormsby, I., Jones-Carson, J., O'Toole, B.A. & Doetschman, T. *Transgenic Res.* **6**, 197–202 (1997).
8. Winstead, C.J., Fraser, J.M. & Khoruts, A. *J. Immunol.* **180**, 7305–7317 (2008).
9. Bommireddy, R. *et al. Lab. Invest.* **86**, 1008–1019 (2006).
10. Marie, J.C., Liggitt, D. & Rudensky, A.Y. *Immunity* **25**, 441–454 (2006).
11. Grossman, Z. & Paul, W.E. *Curr. Opin. Immunol.* **13**, 687–698 (2001).
12. Sprent, J. & Surh, C.D. *Nat. Immunol.* **12**, 478–484 (2011).

Lymph node choreography: B cells take the lead

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The role of T cells in providing help to B cells is well established; however, the converse—that B cells provide signals to help initiate T cell–mediated immunity—is less well appreciated. New data now show B cells modulate the earliest stages of T cell activation in a T helper type 2 response.

Although B cells are recognized mainly for their production of high-affinity antibodies, there is growing appreciation that B cells also have more central roles in orchestrating immune responses. The dynamic interaction between T lymphocytes and B lymphocytes has come to the forefront in studies of follicular helper T cells (T_{FH} cells) showing that B cells have an active role in the development and maintenance of T_{FH} cells. However, a growing body of data indicates that B cells are required for the activation of other T cell responses, most notably the induction of T helper type 2 (T_H2) cells, but also the induction of memory $CD4^+$ T cells[1]. In this issue of *Nature Immunology*, Lund and colleagues show that B cells modulate the earliest stages of T cell activation in a T_H2 response–inducing infection by facilitating the recruitment of mature antigen-presenting dendritic cells (DCs) and $CD4^+$ T cells to regions of the lymph node in close proximity to B cell follicles[2].

According to a now-accepted paradigm, the DC is the critical antigen-presenting cell for the priming of T cell responses, which occur in the T cell zone of lymphoid organs. Naive T cells that express the chemokine receptor CCR7 are recruited into and enter lymph nodes via the high endothelial venules; there, they interact with $CCR7^+$ antigen-presenting DCs in the T cell zone and promote the activation of T lymphocytes. Activation leads to lower CCR7 expression and induction of the chemokine receptor CXCR5 on T cells, which allows their migration to the interfollicular region adjacent to B cell follicles. There, secondary interactions with B cells are required for the formation and maintenance of T_{FH} cells, which support the generation of germinal centers[3]. However, a variety of data now support the view that interactions with B cells contribute not only to T_{FH} cells but also to multiple T cell fates[1]. The interpretation of some experiments in this field, however, has been complicated by the use of B cell–deficient mice, which have defects in the homeostasis of the immune system, including fewer T cells and loss of follicular dendritic cells, as well as aberrant lymphoid organogenesis and splenic microarchitecture[4]. Nonetheless, the importance of B cells as regulators of cellular immune responses has been highlighted by the use of B cell–depleting antibodies in autoimmunity and the observation that some patients benefit, even when the abundance of circulating autoantibodies is not diminished[5].

Lund and colleagues add a new twist to this field of study through the use of *Heligmosomoides polygyrus*, an intestine-dwelling nematode that naturally infects mice and establishes chronic infection[2]. Infection with nematodes remains a major worldwide health problem, with over 1 billion people infected, that results in considerable morbidity. Although resistance is controlled by the induction of robust T_H2 responses, how these responses are initiated is not fully understood. Previous data suggested that B cells take a central role in the initiation and control of these T_H2 responses. Passive transfer of high-affinity immunoglobulin G1 (IgG1) from mice infected with *H. polygyrus* results in a lower parasite burden in newly infected mice[6]; however, such protection does not occur when such antibodies are transferred into B cell–deficient hosts[7]. Furthermore, both T cell–B cell cognate interactions and production of the cytokine interleukin 2 (IL-2) by B cells are integral for a robust T_H2 response independently of antibody production by B cells[7]. Therefore, in addition to producing pathogen-specific antibodies, B cells contribute to other aspects of the immune response.

Expanding on those findings, Lund and colleagues now demonstrate that the generation of IL-4-producing T_H2 and T_{FH} cells in response to infection with *H. polygyrus* occurs in a manner dependent on B cells, lymphotoxin, the chemokine CXCL13 and CXCR5, surprisingly in niches adjacent to the B cell follicle[2]. The authors first show that infection with *H. polygyrus* leads to the induction of CXCR5 on mature DCs and the recruitment of both DCs and $CD4^+$ T cells to areas surrounding B cell follicles (**Fig. 1**). This finding contrasts with the mainly T cell–zone localization of these cells in response to infection with influenza. In a series of elegant experiments with transgenic mice, reconstituted bone marrow chimeras and *in vivo* antibody blockade (to bypass the problems of B cell–deficient mice), the authors demonstrate that the absence of CXCR5 on either DCs or $CD4^+$ T cells impairs the localization of DCs and T cells and negatively affects the generation of both T_{FH} cells and T_H2 cells. The authors further demonstrate that after infection with *H. polygyrus*, B cells secrete lymphotoxin, which leads to more production of CXCL13, presumably from surrounding stromal cells[2] (**Fig. 1**). Either blockade of lymphotoxin or depletion of B cells in *H. polygyrus*–infected mice results in fewer *Cxcl13* transcripts, with

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