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RESEARCH HIGHLIGHT

A two-hit model of autoimmunity: lymphopenia and unresponsiveness to TGF-β signaling

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The immune system can robustly respond to pathogens, while it is capable of maintaining tolerance to self and innocuous antigens, so that the body is not jeopardized by autoimmune reactivity. Under certain circumstances, nonetheless, control of homeostasis of the immune system is suspended, and the body subsequently fails to recognize its own constituent parts as self, which contributes to homeostatic peripheral expansion and autoimmune diseases. Although autoimmunity is extensively studied, the origins of this increasing public health challenge remain not well understood.

In the July issue of *Nature Immunology*, Zhang and Bevan revealed a novel perspective on the potential mechanisms of autoimmune disease and the control of lymphocyte homeostasis and tolerance.[1] Their study provides new experimental support for the two-hit model of autoimmunity that has been previously proposed.[2] By employing mouse models of T cell-specific conditional knockout of transforming growth factor-β (TGF-β) receptor (TGF-βRII), the authors demonstrated that the onset of autoimmune disease requires a trigger such as lymphopenia, but that this lymphopenia-driven autoimmunity can be inhibited by the action of TGF-β signaling on T cells.

TGF-β is a pleiotropic cytokine with diverse effects on hematopoietic cells in the immune system, such as regulating T-cell proliferation, differentiation and survival, and hence maintains immune tolerance and homeostasis.[3] Therefore, the attempt of the current study to understand the likely role of TGF-β in autoimmunity, during which T-cell homeostasis loses control, may open a window for treatment of autoimmune diseases.

To achieve that purpose, firstly, the authors utilized genetic tools to create two different mouse models: early-staged and late-staged conditional knockout of TGF-βRII, respectively. These two knockout systems were accomplished by cross-breeding mice bearing *lox*P-flanked TGF-βRII and mice bearing Cre under the control of the *Cd4* promoter or the distal *Lck* promoter (dLck), which are turned on at early stage and at late stage during lymphocyte development, respectively.

Deletion of TGF-βRII existed in mature T cells in both CD4-Cre and dLck-Cre TGF-βRII knockout mice, from both of which T cells were skewed toward an effector ($CD44^{hi}CD62L^{-}$) or memory ($CD44^{hi}CD62L^{+}$) phenotype.[1,4] Interestingly, the authors found a dramatic difference between these two mouse models. CD4-Cre TGF-βRII knockout mice displayed extensive lymphoproliferation and severe autoimmunity and could live only for 3–5 weeks, while none of the above were observed in dLck-Cre TGF-βRII knockout mice which lived much longer. This raises the question whether the temporal difference in deletion of TGF-βRII caused the intriguing outcome.

The authors addressed that question by looking into the differential deletion of TGF-βRII in newborn mice of the two models, in which neonatal lymphopenia occurred. Lymphopenia, during which the immune system suffers a considerable lack of T cells, is a phenomenon of immune insufficiency that was reported to contribute to autoimmunity.[5] Indeed, they found that on peripheral T cells of neonatal, but not adult, dLck-Cre TGF-βRII knockout mice, TGF-βRII was not efficiently deleted; conversely, TGF-βRII was always deleted in T cells of CD4-Cre TGF-βRII knockout mice. This finding suggests that massive proliferation of TGF-βRII-deficient T cells and subsequent onset of autoimmune disease must happen during the neonatal period in which neonatal lymphopenia provides available space for compensatory homeostatic expansion. In other words, onset of autoimmunity requires two insults to hit together: lymphopenia and the absence of responsiveness of T cells to TGF-β signaling, i.e., the two-hit model of autoimmunity (Table 1).[2]

As adult dLck-Cre TGF-βRII knockout mice do not develop autoimmunity, are the TGF-βRII-deficient T cells derived from those mice still capable of inducing autoimmunity when it is triggered by different lymphopenia other than neonatal lymphopenia? The answer to this question will also reveal to what extent this two-hit model of autoimmunity is supported. To solve this mystery, the authors established two adoptive transfer systems: a $Rag1^{-/-}$ host, which suffers from genetically-induced lymphopenia, and a sublethally irradiated wild-type host, which suffers from experimentally-induced lymphopenia. Naive dLck-Cre TGF-βRII-deficient T cells from adult mice were transferred, either together with control T cells or separately, into $Rag1^{-/-}$ recipient mice. Massive lymphocytic infiltration in the liver and small intestines and greater weight loss were observed in $Rag1^{-/-}$ mice that received TGF-βRII-deficient T cells. In addition, rapid lymphoproliferation and enhanced effector phenotypes and functions were found in

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Table 1 Two-hit model of autoimmunity: lymphopenia and loss of homeostatic control

		Lymphopenia (neonatal/genetic/experimental)	*Loss of homeostatic control (unresponsiveness of T cells to TGF-β in Zhang & Bevan's study)*	*Onset of autoimmunity*
Neonatal, TGF-βRII KO	CD4-Cre, early-staged	+	+	Yes
	dLck-Cre, late-staged	+	−	No
	grow into adult	−	+	No
Adult, *Rag1*$^{-/-}$	+ control T cells	+	−	No
	+ TGF-βRII-deficient T cells (dLck-Cre)	+	+	Yes
Adult, irradiated	+ control T cells	+	−	No
	+ TGF-βRII-deficient T cells (dLck-Cre)	+	+	Yes

Abbreviations: dLck, distal *Lck* promoter; KO, knockout; TGF-β, transforming growth factor-β.

those TGF-βRII-deficient T cells after the adoptive transfer, compared to the control T cells. More importantly, the rapid lymphoproliferation was driven by weak TCR stimuli from self-antigens, i.e. autoimmunity, evidenced by the experiments with antibiotics treatment or adoptive transfer of OT-1 T cells to rule out the involvement of "non-self" antigens. Likewise, rapid lymphoproliferation and autoimmunity occurred to the irradiated wild-type mice that received TGF-βRII-deficient T cells. These discoveries indicate that most likely any lymphopenia, whether neonatal, genetically-induced or experimentally-induced, are capable of triggering autoimmunity, given the loss of homeostatic control, such as unresponsiveness of T cells to TGF-β signaling as in this study (Table 1).

Furthermore, it was revealed that under lymphopenic conditions, TGF-βRII-deficient $CD4^+$ T cells alone were able to induce autoimmunity *in vivo* on their own, whereas TGF-βRII-deficient $CD8^+$ T cells required help from $CD4^+$ T cells. In addition, the *in vitro* finding of the current study that TGF-β signaling considerably inhibited proliferative response of TGF-βRII-deficient $CD8^+$ T cells to weak TCR stimuli, rather than strong TCR stimuli, supplies informative insight into the mechanisms of the T cell-negative and -positive selection and autoimmune disease in which the body responds to self-antigens, as it is believed to be a process of low-affinity TCR recognition from T cells that survive positive and negative selection during thymopoiesis.

As the authors pointed out, autoimmune diseases are often associated with lymphopenia, which is observed in rheumatoid arthritis, insulin-dependent diabetes mellitus, Crohn's disease, systemic lupus erythematosus, primary vasculitides, HIV infection and Sjogren's syndrome.[6] The current findings discussed here agree with the previously proposed two-hit model of autoimmunity.[2] Similarly to the Knudson hypothesis of proto-oncogene and tumor suppressor gene in carcinogenesis process, this two-hit model is composed of a trigger for autoimmunity, as exemplified by lymphopenia, and the loss of control on homeostasis (Table 1). This current study provides a concrete example of the loss of homeostatic control, i.e., the absence of responsiveness of T cells to TGF-β signaling.

In addition to TGF-β unresponsiveness, other factors that can contribute to the loss of control on lymphocytic homeostasis may play similar roles as part of the two-hit model of autoimmunity. These factors include excessive tissue inflammation as seen in HIV-infected patients who develop immune reconstitution inflammatory syndrome and depletion of regulatory T (T_{reg}) cells as demonstrated in murine colitis and gastritis models.[2]

To address the possibility that T_{reg} cells may play a role, the authors cited two previous experiments using the CD4-Cre TGF-βRII knockout mouse model which indicate that T_{reg} cell function might not contribute to the mechanism behind these observations. These studies attempted to reconstitute potentially lacking T_{reg} populations in these mice, which would be lacking due to loss of TGF-β signaling, an important pathway in T_{reg} differentiation from naive T cells. Adoptive transfer of T_{reg} cells directly into CD4-Cre TGF-βRII knockout mice, or generation of mixed-bone marrow chimeras using a mixture of wild-type and CD4-Cre TGF-βRII knockout bone marrow cells failed to prevent autoimmunity.

While T_{reg} cells are clearly not the whole story, their role in this system cannot be completely ignored. Multiple mechanisms have been hypothesized for the suppressive actions of T_{reg} cells, and it is very likely that more than one mechanism is involved in their activity. One of the major mechanisms attributed is the production of TGF-β by T_{reg} cells themselves.[7] Due to lack of TGF-βRII, the CD4-Cre TGF-βRII-deficient T cells are then likely to be highly resistant to T_{reg} activity that might have suppressed wild-type cells in these systems. Similarly, while dLck-Cre TGF-βRII-deficient T cells would have been largely unaffected in the authors' $Rag^{-/-}$ and sublethally irradiated host models, a portion of control T cells may have become T_{reg} cells and in turn suppressed the control T cells in these mice, but not the TGF-βRII-deficient T cells. As one of the important sources of TGF-β, T_{reg} cells are a critical cell type that could potentially be involved in the two-hit model of autoimmunity, since their absence could represent a loss of homeostatic control. Indeed, the predisposition to autoimmunity caused by FoxP3 deficiency, where T_{reg} cell function is lost, is well characterized.[8,9]

Taken together, the current study discloses a critical part of the mystery of autoimmunity and opens new avenues of research to better understand the roles of TGF-β, and possibly T_{reg} cells, in autoimmunity.

1 Zhang N, Bevan MJ. TGF-beta signaling to T cells inhibits autoimmunity during lymphopenia-driven proliferation. *Nat Immunol* 2012; **13**: 667–673.
2 Krupica T Jr, Fry TJ, Mackall CL. Autoimmunity during lymphopenia: a two-hit model. *Clin Immunol* 2006; **120**: 121–128.
3 Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 2006; **24**: 99–146.
4 Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. *Immunity* 2006; **25**: 455–471.
5 King C, Ilic A, Koelsch K, Sarvetnick N. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell* 2004; **117**: 265–277.
6 Schulze-Koops H. Lymphopenia and autoimmune diseases. *Arthritis Res Ther* 2004; **6**: 178–180.
7 Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by $CD4^+CD25^+$ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med* 2001; **194**: 629–644.
8 Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA *et al.* Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001; **27**: 68–73.
9 Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 2003; **15**: 430–435.