Commentary

Natural T cells: Cranking up the immune system by prompt cytokine secretion

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The report by Wilson and colleagues in this issue of PNAS (1) is one of several in immunology that epitomizes the dictum "to understand the physiology of an immunocyte, learn how this cell regulates its gene expression pattern." The study of gene regulation, albeit not new, has shifted from its focus on small numbers of model genes under global control mechanisms to a more comprehensive understanding of gene expression changes within a cell. Despite technical challenges, exciting new insights into mechanisms of gene regulation have emerged from these studies. Recent advances in our knowledge of complex genomes and their expressivity, especially those of mice and humans, coupled with the advent of DNA microarray technology have overcome many past limitations. Thus, a comprehensive analysis of gene expression in cells of identical lineage derived under different conditions, healthy or diseased, resting or activated, progenitor/precursor or differentiated/mature, et cetera, is now possible. Reams of data emerge from a microarray analysis at short notice, the sense of which lies in the mind of the beholder.

Wilson and colleagues (1) have compared the transcriptional consequences within two human natural T cell (referred to in their paper as Vα24JαQ T cell) clones isolated from monozygotic twins discordant for type 1 diabetes and IL-4 secretion. This comparison entailed a comprehensive analysis of their mRNA expression profile with gene chips. One clone was isolated from the normal twin; it elicits IL-4 on activation through its antigen receptor (Tcr). The other clone was derived from the former's identical but diabetic sibling; it is unable to secrete IL-4 (IL-4-null) on Tcr-mediated activation. The results suggest that the natural T cells from the diabetic twin had dysregulated its genes such that it was polarized toward a Th1 phenotype. Additionally, it had shut off or significantly lowered the expression level of several cytokine/chemokine genes whose products are normally involved in recruitment, activation, and differentiation of dendritic cells and macrophages.

Natural T cells (alias NK1$^+$ T cells or NKT cells) are a subset of thymus-derived lymphocytes that express phenotypic markers typical of natural killer (NK) cells and T cells. They include T lymphocytes of CD4$^+$ and CD4$^-$/8$^-$ double-negative phenotype that express a conserved Tcr α-chain. In humans, they express Vα24JαQ whose CDR3α loop, the region that contributes significantly to the antigen-receptor interface (2), is highly conserved. This conserved Tcr α-chain pairs predominantly with Vβ11. Their homologues Vα14Jα15 (formerly Jα281) and Vβ8.2 are expressed in mouse natural T cells; a small proportion of these cells in mice also pairs with Vβ7 and Vβ2 (for a comprehensive review, see ref. 3).

The physiological role of natural T cells, albeit elusive, is thought to be immunoregulatory in nature (3). This function is controlled by CD1d (4, 5), an evolutionarily conserved glycolipid antigen-presenting molecule (6). Natural T cells have several unique functional characteristics that deserve comment. They are among leukocytes that, on activation through crosslinking of their antigen receptors *in vivo*, promptly secrete large amounts of IL-4 without prior priming with this cytokine (7). IL-4 is an immunomodulatory cytokine that polarizes newly activated CD4$^+$ helper T (Th) cells toward Th2 effector function (8). Several lines of evidence have suggested a link between natural T cell deficiency or dysfunction and autoimmune diseases (9–13). The best studied of these disorders is insulin-dependent diabetes mellitus (type I diabetes). Both humans and mice predisposed to or afflicted by type 1 diabetes have fewer natural T cells, and those present are dysfunctional, in that they are unable to promptly secrete IL-4 in response to crosslinking of their antigen receptors (9, 13). In nonobese diabetic (NOD) mice, the mouse model for human type 1 diabetes (14, 15), adoptive transfer of thymic natural T cells into prediabetic animals corrected the deficiency and the disease (11). Protection from disease by adoptive transfer of natural T cells required IL-4 and/or IL-10 (11). Additionally, administration of recombinant IL-4 itself (16) to prediabetic NOD animals prevented them from developing diabetes. Thus, an IL-4-mediated immunoregulatory role *in vivo* is ascribed to natural T cells.

The results reported by Wilson *et al.* (1) revealed that the natural T cell from the diabetic sibling is transcriptionally dysregulated such that its gene expression pattern was consistent with polarization toward a Th1 phenotype. This gene expression pattern is distinct from the nondiabetic twin's natural T cells, which expressed a pattern reflective of Th2 phenotype. Specifically, of the 6,800 genes represented within the DNA microarray, about an equal number of expressed genes ($\approx$1,500) from the two natural T cell clones were detectable under resting and activated conditions. Amongst these, 226 and 86 transcripts were significantly modulated within the IL-4-secreting and IL-4-null natural T cell clones, respectively, on activation. Of the modulated transcripts, only 28 were shared between the two clones. Interestingly, the expression of transcription factors and signaling molecules were among those that were differently regulated between the two natural T cell clones. Transcription factors that bestow the Th2 phenotype on CD4$^+$ cells (e.g., GATA3 and JunB; refs. 17 and 18) were up-regulated in the activated IL-4-secreting clone. In contrast, signaling molecules (e.g., STAT1, STAT4, and CD161) involved in transducing Th1 cytokine signals (e.g., IFN-γ and IL-12; refs. 19–22) were up-regulated in the IL-4-null clone. NFAT4, a transcription factor thought to suppress IL-4 transcription (23), was overexpressed in the IL-4-null clone, consistent with its inability to secrete IL-4. These differences explain why normal natural T cells promptly secrete large amounts of IL-4 on *in vivo* activation. Additionally, they also explain how natural T cells in diabetics may be dysregulated and attain a Th1 phenotype.

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Table 1. Properties of cytokines/chemokines elicited by natural T cells

Cytokine/chemokine	Source: Natural T cell		Target cells	Function(s)‡
	Nondiabetic*	Diabetic†		
IL-2	+		Lymphocytes	Autocrine growth factor
IL-4	+++++	−	Leukocytes	Antiinflammatory; Th2 and B cell activation and differentiation; immunoglobulin isotype switching to IgE; eosinophilia
IL-5	++	−	Eosinophils	Eosinophilia
IL-13	+++++	−	Leukocytes	Antiinflammatory; Th2 and B cell activation and differentiation; immunoglobulin isotype switching to IgE
IFN-γ	+++	+	Leukocytes	Proinflammatory; host defense against tumors and viruses; macrophage, NK, and cytotoxic T lymphocyte activation
GM-CSF	+++++	+	Myeloid cells	Hematopoiesis; activation and differentiation of myeloid cells
TNFα	++	−	Leukocytes Endothelial cells	Proinflammatory; local inflammation; activation of endothelial cells
TNFβ	++	−	Leukocytes Endothelial cells	Proinflammatory; cytotoxic; endothelial cell activation
MIP-1α	+++++	+	Leukocytes	Chemoattractant; activation
MIP-1β	+++	+	Leukocytes	Chemoattractant; activation
Lymphotactin	+++	−	Lymphocytes	Chemoattractant

GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor; MIP, macrophage inflammatory protein.
*Refs. 1 and 3.
†Ref. 1.
‡Refs. 35 and 36.

α-Galactosylceramide (α-GalCer), a potent synthetic glycolipid antigen presented by CD1d, activates natural T cells *in vivo*, resulting in the secretion of both IFN-γ and IL-4 (24–26). Both cytokines are elicited promptly in response to α-GalCer; however, IFN-γ is maintained only acutely, whereas IL-4 is maintained chronically (26). The chronic maintenance of IL-4 in the milieu of differentiating Th cells polarizes them toward Th2 phenotype (26). Moreover, the IFN-γ response to α-GalCer seems to be elicited by NK cells requiring IL-12-mediated activation (27–29). IL-12 is a proinflammatory cytokine elicited by activated myeloid cells such as dendritic cells and macrophages (30). How activation of natural T cells by α-GalCer results in IL-12 production is currently unclear.

Wilson and colleagues (1) report that, in contrast to the IL-4-secreting natural T cell, activation of the IL-4-null clone induced at best extremely low levels of only a few cytokine/chemokine transcripts. The IL-4-secreting natural T cell transcribed high levels of messages for cytokines/chemokines important for the recruitment, activation, and differentiation of macrophages and dendritic cells (Table 1). The proinflammatory factors such as GM-CSF, MIP-1α, and MIP-1β promote the recruitment, activation, and/or differentiation of dendritic cells and macrophages (Fig. 1; refs. 31 and 32). Activated dendritic cells and macrophages secrete IL-12 and/or other factors that stimulate NK cells, T cells, and possibly natural T cells to secrete IFN-γ (30), explaining how α-GalCer induces IFN-γ secretion by NK cells and possibly natural T cells in an IL-12-dependent manner.

Thus, natural T cells on activation *in vivo* promptly secrete numerous cytokines that include proinflammatory and antiinflammatory factors (Table 1) without the need for prior sensitization to these factors. The prompt elicitation of cytokines/chemokines in response to antigen probably helps jumpstart the innate and adaptive components of the immune system (Fig. 1). The Th1-inducing cytokines produced for a short period and IL-4 secretion sustained for a longer period have the potential for stimulating both the cellular and humoral limbs of the adaptive immune system. However, the IL-4 burst on activation of natural T cells by antigen counter regulates Th1-promoting cytokines and helps Th2 differentiation and further elaboration of IL-4; thus, IL-4 is sustained *in vivo* in a chronic manner and hence promotes Th2-mediated immunity.

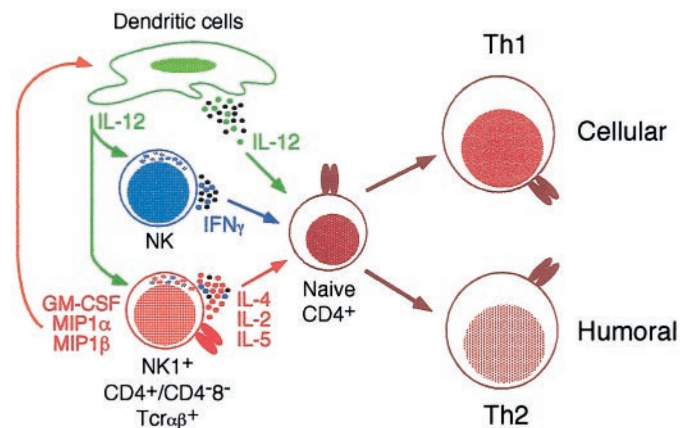

Fig. 1. A model for natural T cells' role within the immune system. Natural T cells participate in cross talks between members of the innate and the adaptive immune systems by deploying cytokine/chemokine messengers. Current data suggest that natural T cells on activation *in vivo* promptly secrete several cytokines/chemokines. Of these cytokines/chemokines, IL-4 can skew the differentiation of CD4$^+$ T cells toward the Th2 phenotype. GM-CSF, MIP-1α, and MIP-1β recruit, activate, and differentiate macrophages and dendritic cells resulting in the production of IL-12 and possibly other factors. IL-12, in turn, stimulates NK cells and possibly natural T cells to secrete IFN-γ. Along with IL-12, IFN-γ can polarize the differentiation of antigen-activated CD4$^+$ T cells toward the Th1 phenotype. Thus IL-4, GM-CSF, MIP-1α, and MIP-1β can be thought of as primary cytokines, and IFN-γ can be thought of as a secondary cytokine of natural T cells. The Th1 cytokines can counterbalance Th2 and vice versa. How this counterbalance is accomplished under conditions where both Th1 and Th2 cytokines elicited by natural T cells are present simultaneously remains unclear. (See text for references.)

Among other differences observed between the IL-4-secreting and IL-4-null natural T cells were genes activated through the phosphatidylinositol-3 kinase pathways important for calcium flux and cell survival (e.g., PLCγ1, Itk, BCL_{XL}, and IAP) as well as cytokine response (1). Curiously, genes induced through the phosphatidylinositol-3 kinase-dependent pathway were expressed at a higher level within the activated IL-4-secreting clone (1). Given the results of previous studies in mice demonstrating rapid turnover of natural T cells within the first few hours of activation through their Tcr or by IL-12 (33), it is surprising that the cell survival genes were up-regulated in the IL-4-secreting cells. Thus, the converse would have been predicted, especially because natural T cells from NOD mice are resistant to activation-induced cell death (34). This disparity could have arisen from using clones maintained *in vitro*, because the studies in mice were conducted *in vivo* in one instance (33) and *ex vivo* in the other (34).

In summary, gene chip-based DNA array technology provides a powerful tool for a comprehensive analysis of gene regulation. This approach by Wilson and colleagues (1) has provided insights into the mechanism of how the IL-4-null and the IL-4-secreting natural T cell clones might attain Th1- and Th2-like phenotype, respectively. Their approach opens the door to address several outstanding questions regarding the role of natural T cells in autoimmune diseases: (*i*) Does the pattern of gene regulation observed for clones maintained *in vitro* reflect that of *in vivo* activated natural T cells? (*ii*) Is the Th1-like character of natural T cells the cause or the consequence of type 1 diabetes? (*iii*) What determines the Th0 (possessing both Th1 and Th2 cytokine profile) versus Th1 fate of natural T cells in healthy and autoimmune disease states? Answers to these questions should be forthcoming from a comprehensive analysis of gene regulation during natural T cell development and differentiation *in vivo*.

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