γδ T Cell Receptors without a Job

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In this issue of *Immunity*, the studies by Sutton et al. (2009) and Martin et al. (2009) indicate that γδ T cells are innate cells that rapidly produce interleukin (IL)-17 in response to cytokines or pathogens without the need for T cell receptor engagement.

There are compelling reasons to consider the notion that T cells that express the gamma-delta T cell receptors (γδ TCRs) are innate cells that critically contribute to host defense. First of all, in contrast to conventional αβ TCRs and similarly to innate receptors, the repertoire of γδ TCRs is very limited and directly recognizes poorly defined polymorphic structures without the requirement for processing and presentation by specialized antigen-presenting cells. In addition, γδ T cells are found in small numbers in lymphoid tissues but are more abundant in peripheral tissues. Another striking feature shared with innate cells is that within hours upon infection, antigen-naive γδ T cells already produce high amounts of effector cytokines, including IL-17 and interferon (IFN)-γ that contribute to early recruitment of more specialized innate effectors, functional polarization of αβ T cells, and/or direct clearance of invading pathogens (Roark et al., 2008). In particular, IL-17 is linked to protection against bacterial and fungal infections through the recruitment of neutrophils (Korn et al., 2009) and protection against type 1 $CD4^+$ T helper (Th1) cell-mediated pathology in chronic inflammatory disease (O'Connor et al., 2009). Whereas the concept of IL-17 production by αβ Th (Th17) cells and the conditions of their development warranted much attention over the last few years, the role of IL-17-producing γδ T (γδ T-17) cells remained in the background. In fact, an overwhelming majority of IL-17-producing cells in mice are made up of γδ T cells (Awasthi et al., 2009), and these cells are critically protective in many mouse models of infection and chronic inflammation (O'Brien et al., 2008). γδ T cells are heterogeneous, not only in terms of their polymorphic γδ TCRs and their localization but also in terms of their effector cytokine profile. It was previously shown that antigen-naive mice harbor ($CD25^+$ $CD122^{lo}$) IL-17- and ($CD122^{hi}$) IFN-γ-producing γδ T cell subsets of which the functional segregation is predetermined during their development in the thymus (Shibata et al., 2008; Jensen et al., 2008).

A confusing issue is that upon infection of antigen-naive mice γδ T cells are, without prior expansion, massively triggered to rapidly produce IL-17, whereas the pathogen-derived ligands are mainly unknown. In this issue of *Immunity*, Mills and colleagues (Sutton et al., 2009) and Veldhoen and colleagues (Martin et al., 2009) propose an unexpected answer to this question, by suggesting that, in contrast to αβ Th17 cell function, γδ T cells can be triggered to produce IL-17 upon innate signals without TCR ligation. The many recent studies on αβ Th cells have revealed that the development of αβ Th17 cells requires antigen-specific stimulation of naive Th cells in the presence of Th17 cell-polarizing cytokines produced by antigen-presenting dendritic cells or bystander cells (Korn et al., 2009). This Th17 cell development is initiated in naive Th cells by the cytokines TGF-β and IL-6 (and autocrine IL-21), which induce the expression of IL-23 receptor (IL-23R), chemokine receptor CCR6, and the Th cell subset-specific transcription factor RORγt, which is necessary and sufficient for induction of IL-17. However, these Th17 cells have to become stable inflammatory effector cells through the additional activation by the cytokines IL-23 and IL-1. Pathogens can induce the production of these cytokines in many host cell types upon the triggering of selected pattern recognition receptors (PRRs). IL-23 and IL-1 may be selectively triggered by bacterial lipids ligating Toll-like receptor (TLR) 2 and by fungal β-glucane triggering dectin 1. The surprising picture drawn by the two present studies is that the induction of IL-17 production by γδ T cells can be strikingly different from that of αβ Th cells. They show that the subset of γδ T-17 cells display an activated phenotype, express IL-23R, CCR6, and RORγt, and are able to rapidly produce IL-17A (as well as IL-17F, IL-21, and IL-22) upon immunization in vivo, features that resemble antigen-experienced αβ Th17 cells (Figure 1). Most strikingly and in sharp contrast to IL-17 production by effector Th17 cells, IL-17-production by T cells does not need TCR engagement but can be merely triggered by the cytokines IL-23 and IL-1, as produced by pathogen-activated dendritic cells as bystander cells (Sutton et al., 2009) or by the direct triggering of TLR2 and dectin-1 by pathogens (Martin et al., 2009).

In line with a previous study showing that the maintenance of γδ T-17 cells was severely compromised in the absence of IL-23 but not IL-6 (Lochner et al., 2008), the study by Mills and colleagues (Sutton et al., 2009) shows that the combination of IL-23 and IL-1, and not IL-6 and TGF-β, induce IL-17 in γδ T cells and that these cytokines are instrumental as products of cocultured dendritic cells activated by killed bacteria (i.e., *Mycobacterium tuberculosis*). An initial unique finding of this study was that effector cytokine production by γδ T cells did not require TCR ligation by anti-CD3, albeit that the amounts of IL-17 were substantially lower in the absence of anti-CD3. They subsequently applied a mouse model of experimental encephalomyelitis (EAE), which is known to depend on brain antigen (MOG)-specific T cells and IL-17. After immunization with MOG

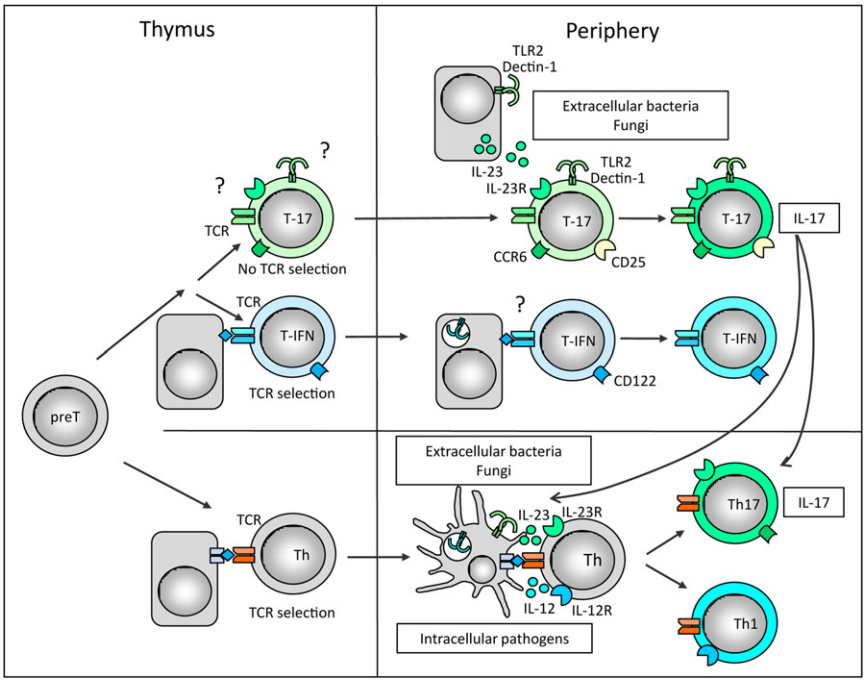

Figure 1. Innate Activation of IL-17-Production by a Specialized γδ T Cell Subset
Engagement of the T cell receptor (TCR) of αβ T cells selects their repertoire in the thymus and allows them to respond in the periphery to signals that polarize their function, such as the cytokine IL-23, which stabilizes IL-17 production in T cells, or IL-12, which promotes IFN-γ production (bottom left and right). Antigen-naive mice γδ T cells, however, harbor $CD122^{lo}CD25^{hi}$ γδ T (γδ T-17) cells that, in contrast to αβ T cells and $CD122^{hi}$ γδ T cells (that contain IFN-γ producers), are predetermined in the thymus to become IL-17 producers without TCR engagement (top left). New studies (Sutton et al., 2009; Martin et al., 2009) underscore TCR independency of γδ T-17 cells, also at their functional level (top right, green cells). They show that γδ T-17 cells additionally express the receptor for IL-23, and the innate receptors TLR2 and dectin-1, which recognize archetypical bacterial and fungal constituents. These γδ T-17 cells use these receptors to rapidly produce IL-17 in response to bystander cell-derived IL-23 and to bacteria and fungi without concomitant TCR stimulation. One of the roles of γδ T-17-derived IL-17 may be indirect (via potentiation of IL-23 production in antigen-presenting dendritic cells) or direct amplification of IL-17 production in Th17 cells (curved arrows).

in complete Freunds' adjuvant (containing mycobacterial compounds) and during clinical symptoms, they found enhanced amounts of IL-1 and IL-23p19 mRNA that coincided with elevated IL-17 mRNA expression and the detection of γδ T-17 cells during the development of EAE. A contribution of the γδ T-17 cells to EAE pathology was substantiated by experiments in mice lacking γδ T cells or IL-1 receptors, with delayed onset of EAE and less severe symptoms for the mice, and by transfer experiments. Moreover, these transfer experiments revealed that cooperation between γδ T-17 cells and $CD4^+$ T cells is vital for EAE development. This observation inspired a subsequent series of in vitro and in vivo experiments indicating that IL-23 and IL-1 promote the innate production of IL-17 by γδ T cells and that IL-17 subsequently amplifies IL-17 production by antigen-specific Th17 cells, either by the direct interaction with $CD4^+$ T cells or, indirectly, by amplifying IL-23 production by dendritic cells. The data suggest that γδ T-17 cells have a pathogenic role early in EAE development, among others by controlling the generation of Th17 cells. The control of Th17 cell function by γδ T-17 cells is an unexpected and attractive concept that requires further confirmation.

Veldhoen and colleagues (Martin et al., 2009) detected early γδ T-17 cells in mice, in particular after immunization with *M. tuberculosis* or *C. albicans* hyphae or with bacterial lipid TLR2 agonist (and not with TLR4 agonist) and with fungal β-glucane, the dectin-1 agonist. They share with the study by Mills and colleagues the finding that γδ T-17 T cells produce IL-17 in absence of γδ TCR ligation. However, elaborating on previous reports on the expression of TLR2 on γδ T cells, they show that only $CCR6^+$ γδ T cells express TLR2 and/or dectin-1 and produce IL-17 upon direct ligation by TLR2 and dectin-1 agonists and that this IL-17 production is amplified by IL-23. Interestingly, their experiments showed that mere IL-23 addition also amplifies IL-17 production, even more strongly than TLR2- or dectin-1 ligation, and that the combination of IL-23 and innate receptor ligation was best. Transfer of γδ T cells into TLR2-deficient hosts challenged with a bacterial TLR2 agonist elegantly underscored direct pathogen recognition by γδ T-17 cells in vivo.

Taken together, the two present studies highlight the innate nature of the γδ T-17 cell subset by showing its selective expression of IL-23R, TLR2, and dectin-1 and its responsiveness to IL-23 and IL-1 produced by accessory cells activated by bacterial and fungal infection as well as its direct responsiveness to bacterial TLR2- and fungal dectin-1 agonists. γδ T-17 cells act like other innate immune cells by directly responding to pathogens or to cytokines produced by cells in infected tissue and, as a result, producing cytokines that amplify the function of other innate or adaptive immune cells. A striking consequence of the new findings is that the role of γδ TCRs in γδ T cell-mediated host defense may be redundant. The role of γδ TCRs in γδ T-17 cell function was already suggested to be redundant at the level of T cell development with the finding that γδ T-17 cells form a unique γδ T cell subset of cells that become predetermined IL-17 producers in the thymus seemingly without prior TCR engagement and, consequently, without positive and negative TCR selection (Jensen et al., 2008). The mechanisms underlying the development of γδ T-17 cells in the thymus and the way these cells transit from a naive into activated phenotype in antigen-naive mice is a mystery. The knowledge that these cells can be activated in the periphery by cytokines and innate signals without TCR engagement may provide alternative approaches to these questions. However, TCR engagement by anti-CD3 on lymph node $CD122^{lo}$ cells does result in IL-17 production (Jensen et al., 2008) even in the absence of IL-23 and IL-1, stressing γδ TCRs can be involved in γδ T-17 function. Therefore, the deciphering of relative

roles of cytokines and PRR and TCR ligands in the development of γδ T cells and their function in protection and pathology is a great challenge for future research.

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