Immunity
Previews

γδ T Cell Selection: Is Anyone Useless?

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DOI 10.1016/j.immuni.2008.06.007

In this issue of *Immunity*, a study by Jensen et al. (2008) suggests that T cell-receptor engagement during development affects γδ T cell polarization toward either interferon-γ or interleukin-17 production. This might underlie their unique innate ability to regulate inflammation.

Lymphocytes expressing γδ T cell receptors (TCRs) display several peculiar features that make them more similar to innate than adaptive immune effectors (Chien and Bonneville, 2006). Indeed, γδ cells use only a tiny fraction of their potentially highly diverse TCR repertoire to recognize a restricted set of conserved stress-induced antigens. Murine γδ cells in skin represent an extreme case of such a repertoire restriction in that they express identical—so-called invariant—γδ TCRs made of Vγ5Vδ1 regions. Even splenic γδ cells, which show biased VγVδ usage but yet highly diverse TCRs, frequently recognize the same weakly polymorphic MHC-related molecules T10 and T22. Besides their ability to recognize a limited set of monomorphic ligands, γδ cells also share with innate effectors the capacity to rapidly exert their effector functions upon Ag encounter, even in naive individuals. This is highlighted by the strong and early production of either pro- or anti-inflammatory cytokines, such as IFN-γ, IL-4, or IL-17, that can control the early recruitment of innate effectors, functional polarization of conventional αβ T cells, and/or direct clearance of the eliciting pathogens (Roark et al., 2008). How the γδ TCR repertoire is generated and how γδ cells acquire their innate functional properties is still unclear. In this issue of *Immunity*, a study by Chien and colleagues brings several new insights into both issues (Jensen et al., 2008).

Differentiation of conventional αβ T cells from thymic precursors involves a succession of developmental checkpoints that allow efficient generation of lymphocytes expressing nonautoreactive, yet self-MHC-restricted TCRs. A first checkpoint, called β selection, allows CD4CD8 double negative (DN) thymic precursors that have produced functional TCR β chains to proceed along αβ differentiation and mature into CD4CD8 double positive (DP) thymocytes. These cells then undergo selection events that involve interaction between αβ TCRs and self-MHC peptide complexes expressed by either epithelial or hemopoietic thymic cells. Positive selection refers to the terminal differentiation of DP thymocytes that have established TCR interactions of intermediate avidity for self-MHC-peptides, whereas negative selection refers to the deletion or anergization of thymocytes expressing TCRs with high affinity for self. Hence, thymic TCR selection permits production of nonharmful but useful T cells, which are able to interact with sufficient affinity with Ags encountered in the periphery. Is there a similar need to select a useful γδ TCR repertoire? αβ and γδ cells actually originate from common thymic precursors, which are committed to either one or the other lineage at the end of the DN stage. Whereas β selection engages DN precursors toward the αβ lineage, expression of a functional γδ TCR triggers their differentiation into mature γδ T cells. β selection and γδ selection are analogous processes that both induce thymocyte proliferation and similar upmodulation or downmodulation of several developmental markers and transcription factors (Taghon et al., 2006). Therefore, like development of αβ cells, γδ development involves epigenetic events that promote survival, expansion, and differentiation of precursors that have successfully produced functional TCR chains. Whether or not γδ selection is a bona fide positive-selection process, involving interactions between γδ TCRs and thymic ligands, is less clear. Early analysis of mice transgenic for a γδ TCR specific for the β2microglobulin (β_2m)-associated T10 and T22 MHC-related molecules suggested efficient generation of fully competent transgenic lymphocytes in the absence of TCR engagement, which is in a β_2m-deficient background (Schweighoffer and Fowlkes, 1996). Hence, γδ cells did not seem to undergo positive selection, but this assumption was hampered by possible artifacts linked to TCR transgenesis. To avoid this caveat, Jensen et al. (2008) directly quantitated and characterized by flow cytometry T10- and T22-specific γδ lymphocytes in non-TCR transgenic mice, with a fluorescent tetramerized T22 reagent able to specifically label these lymphocytes. Thymic and peripheral lymphocytes specific for T10 and T22 molecules showed a more mature phenotype in mice that expressed these Ags than in mice that did not express them, consistent with TCR engagement in the former but not the latter case. However, the frequency and antigenic affinities of thymic and periperhal T22-specific γδ lymphocytes were similar in both backgrounds. Therefore, this study provides compelling evidence that at least some γδ T cell precursors can differentiate into functional γδ cells able to exit the thymus without having to engage their TCRs during development. However, the actual impact of γδ TCR selection on the fine composition of the peripheral γδ repertoire remains unclear. Repertoire shaping by negative selection is suggested by the decreased frequency of high-affinity T22-specific splenocytes in ligand-sufficient mice, when compared to ligand-deficient ones. Moreover, recent studies suggest that generation of skin γδ cells expressing invariant Vγ5Vδ1 TCRs requires positive selection by as-yet-unidentified thymic ligands (Lewis et al., 2006; Xiong et al., 2004). Jensen et al. (2008) propose a possible mechanism

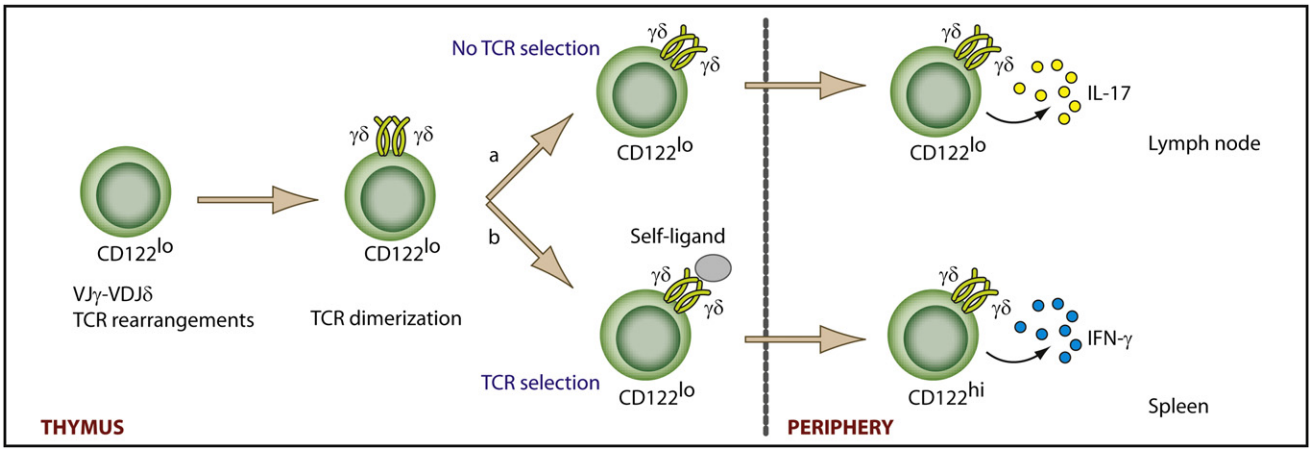

Figure 1. TCR Engagement during Development Determines the Functional Polarization of γδ T Cells into Either IFN-γ or IL-17 Producers
According to a new study (Jensen et al., 2008), expression of a γδ TCR by thymic γδ T cell precursors readily triggers their survival and differentiation into functional γδ T lymphocytes, owing to the ability of most γδ TCRs to spontaneously dimerize and induce signaling without antigen-driven crosslinking. Unselected γδ+ precursors, i.e., which have not engaged their TCRs, acquire the ability to produce pro-inflammatory cytokines (such as IL-17) and accumulate in lymph nodes (pathway *a*). Upon interaction between γδ TCRs and thymic self-ligands, selected precursors are polarized toward IFN-γ secretion and accumulate in the spleen (pathway *b*).

that could reconcile these findings, as they provide indirect evidence that many γδ TCRs, but not invariant Vγ5Vδ1 TCRs, spontaneously form dimers and therefore can signal without ligand engagement. Therefore, TCR dimerization could induce survival, proliferation signals, and thymus exit but not full phenotypic maturation (associated in particular with upregulation of the CD122 developmental marker), which would be only triggered by TCR selection (Figure 1).

Quite strikingly, Jensen et al. (2008) found that splenic γδ cells were predominantly CD122^{hi} and made IFN-γ, whereas lymph-node γδ cells were mainly CD122^{lo} and readily made IL-17, a major inflammatory cytokine, upon TCR crosslinking. Hence, not only do γδ cells in different lymphoid organs yield distinct cytokine responses, but more unexpectedly they also seem to readily acquire pro-inflammatory functions without Ag encounter. In this regard they would substantially differ from αβ cells, whose differentiation into IL-17 producers requires Ag-induced priming. These findings could explain how γδ cells can mount strong IL-17 responses so quickly, and such responses in turn could contribute to neutrophil recruitment, early clearance of some bacterial pathogens, and inflammatory polarization of conventional αβ T cell responses (Roark et al., 2008). However, it is assumed, but not formally proven, that all CD122^{lo} γδ cells in the thymus and peripheral lymphoid organs are truly naive unselected lymphocytes; this assumption might not be necessarily the case because some CD122^{lo} γδ cells, like those located in the gut, display several hallmarks of primed T cells. Therefore, it will be important to confirm this IL-17 expression bias among truly unselected γδ cells, such as T22-specific splenocytes from β_2m-deficient mice.

In any case, these findings raise several questions regarding the mechanisms controlling polarization toward IL-17 or IFN-γ responses along γδ development and the signals that trigger these effector functions in the periphery. Functional programming of αβ lymphocytes into Th17 cells requires expression of the nuclear orphan receptor RORγt (McGeachy and Cua, 2008). RORγt is also highly expressed by DP thymocytes but rapidly turned off in selected thymocytes (He, 2002). Similarly, it might be upregulated upon TCR γδ dimerization and downmodulated only upon γδ TCR engagement, an issue that could be addressed through detailed analysis of RORγt expression along γδ differentiation. It will be also interesting to determine whether IL-17 programming of unselected γδ cells is truly a default pathway or whether it requires additional signals. A way to address this could be through estimation of the frequency of precommitted Th17 cells among unselected γδ cells, e.g., through analysis of IL-23-receptor expression because this receptor is known to be upregulated on primed Th17 lymphocytes. In this respect, IL-23, which is released by antigen-presenting cells infected by bacteria engaging a particular set of innate receptors, can readily trigger IL-17 responses of both primed αβ and naive γδ cells, but such responses are strongly enhanced by TCR crosslinking. Because γδ TCRs show germline reactivity toward ligands upregulated along cell stress and activation, signaling by even unselected γδ TCR could contribute to the fine regulation of IL-17 γδ responses in the periphery and link them to inflammatory contexts associated with upregulation of a restricted set of stress-induced ligands. In conclusion, the study of Jensen et al. (2008) describes a new mechanism that could explain how γδ cells acquire their unique ability to rapidly yield, upon sensing of danger signals, cytokine responses that will contribute to the fine tuning of effector functions mediated by other innate and adaptive immune cells. How precisely such innate properties are acquired and imprinted along γδ development remains unclear, but the hints provided by this new study will certainly help tackle this exciting issue.

REFERENCES

Chien, Y.H., and Bonneville, M. (2006). Cell. Mol. Life Sci. *63*, 2089–2094.

He, Y.W. (2002). J. Leukoc. Biol. *72*, 440–446.

Jensen, K.D.C., Su, X., Shin, S., Li, L., Youssef, S., Yamasaki, S., Steinman, L., Saito, T., Locksley, R.M., Davis, M.M., et al. (2008). Immunity *29*, this issue, 90–100.

Lewis, J.M., Girardi, M., Roberts, S.J., Barbee, S.D., Hayday, A.C., and Tigelaar, R.E. (2006). Nat. Immunol. *7*, 843–850.

McGeachy, M.J., and Cua, D.J. (2008). Immunity *28*, 445–453.

Roark, C.L., Simonian, P.L., Fontenot, A.P., Born, W.K., and O'Brien, R.L. (2008). Curr. Opin. Immunol. *20*, 1–5.

Schweighoffer, E., and Fowlkes, B.J. (1996). J. Exp. Med. *183*, 2033–2041.

Taghon, T., Yui, M.A., Pant, R., Diamond, R.A., and Rothenberg, E.V. (2006). Immunity *24*, 53–64.

Xiong, N., Kang, C., and Raulet, D.H. (2004). Immunity *21*, 121–131.

Regulatory T Cells and Inflammation: Better Late Than Never

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DOI 10.1016/j.immuni.2008.07.001

In this issue of *Immunity*, Curotto de Lafaille et al. (2008) show that adaptive T regulatory cells control airway inflammation and that it is when they are generated that determines whether they function during acute or chronic inflammation.

The airways are constantly assaulted by aeroantigens, yet the vast majority of individuals are tolerant and mount no immune response. However, atopic individuals respond inappropriately to these innocuous aeroantigens and develop airway inflammatory disease (asthma) characterized by remodeling and hyperresponsiveness of the airways. That asthma is a disease of immune dysregulation is exemplified by the fact that affected individuals have an elevated number of T helper 2 (Th2) cells and Th2 cell-type cytokines in the lung as well as elevated amounts of IgE and lung eosinophilia (Wills-Karp, 1999). The ability of the majority of people to remain unresponsive to aeroantigens has been ascribed to the ability of regulatory T (Treg) cells and immunomodulatory cytokines (e.g., IL-10) to control responses (Akdis, 2006; Umetsu and Dekruyff, 2006). Although the role of Treg cells to control airway inflammation has been shown in transfer models, what role they play as disease develops remains to be determined. In addition, how aeroantigen-specific Treg cells, if they exist, develop and function is an area of intense investigation and discussion. The data in Curotto de Lafaille et al. (2008) begin to develop a framework for uncovering the role of Treg cells in both acute and chronic airway inflammation in the lung, and, by extension, other mucosal surfaces.

In naive mice, the outcome of an encounter with an inhaled antigen, in the absence of an inflammatory stimulus, is tolerance, not activation. However, this tolerance is not the result of a lack of a response to the antigen, as shown by the fact that robust $CD4^+$ T cell proliferation is seen in draining lymph nodes after treatment (Hammad and Lambrecht, 2008). Rather, it may be due to incomplete dendritic cell activation, resulting in either aborted T cell activation and subsequent deletion or the generation of T cells with regulatory activity (Hammad and Lambrecht, 2008). These tolerized mice are then resistant to subsequent challenge with the same antigen in the presence of an inflammatory stimulus. In addition, adoptive transfer of antigen-specific Treg cells can inhibit disease development, and depletion of $CD4^+CD25^+$ cells increases disease parameters in challenged mice (Lewkowitch et al., 2005), further supporting an important role for Treg cells in controlling inflammation in airways.

The mechanism by which these Treg cells control airway inflammation is not at all clear. Several reports have suggested that Treg cells block inflammation in an IL-10-dependent manner. For example, either transfer of Treg cells from IL-10-deficient mice or IL-10 blockade after transfer of Treg cells abolished inhibition of airway inflammation (Kearley et al., 2005). In contrast, Treg cells induced by helminth infection are capable of completely inhibiting allergic airway inflammation in an IL-10-independent manner (Wilson et al., 2005).

Several important issues remain to be resolved concerning the role of Treg cells in controlling airway inflammation. These include whether the Treg cells seen in these models are thymically derived, what the role of Foxp3 is in their generation and function, and what role, if any, inflammation plays in controlling Treg cell generation and function. By using a very elegant and simple system, Curotto de Lafaille et al. (2008) provide insight into these issues. They take advantage of mice whose T and B cell repertoires are non-self reactive and monoclonal through expression of a single T cell receptor (TCR) and BCR in a Rag-deficient background (referred to as T-Bmc mice). Similar to other TCR transgenic mice in a Rag-deficient host, these mice lack thymically derived, "natural," Treg cells, and thus can be used to determine the role of adaptive Treg cells in tolerance to aeroantigens. The other advantage of these mice is that they can be rendered Foxp3 deficient without causing the