The cells that knew too much

Alan G. Baxter

Centenary Institute of Cancer Medicine and Cell Biology, Locked Bag #6, Newtown NSW 2042, Australia.
Phone: 61-2-95656174; Fax: 61-2-95656103; E-mail: A.Baxter@Centenary.usyd.edu.au.

Commentary

See related article, pages 1761–1767.

In this issue of the *JCI*, Ikehara et al. (1) report a novel function for an unusual population of lymphocytes — natural killer T (NKT) cells. These cells were originally identified as $CD4^-CD8^-$ (double negative, DN) T cells responsible for rapid production of large amounts of IL-4 (2, 3), a cytokine that plays a critical role in supporting immunoglobulin production and inhibiting some inflammatory responses. NKT cells are now usually identified either by the coexpression of the NK cell marker NKR-P1 (NK1.1 in mice) and the T-cell antigen receptor (TCR), or by the presence of unusually restricted TCRα chains (termed Vα24JαQ in humans and Vα14Jα281 in mice and rats; refs. 4, 5). Although these cells may either be DN or express intermediate levels of the CD4 accessory molecule ($CD4^{int}$), the fact that most NKT cells in humans, mice, and rats use similar TCRα chains has led to the suggestion that they recognize a restricted range of targets (4). Consistent with this hypothesis, many NKT-cell clones appear to be stimulated by the MHC class I–like molecule CD1, and NKT cells are severely depleted in mice carrying a targeted deletion of CD1d (6, 7).

NKT cells in autoimmunity and host defense

NKT cells play an important role in immunoregulation. We and others have found that nonobese diabetic (NOD) mice, which serve as a model of type 1 (autoimmune) diabetes, are deficient in NKT cells in the thymus (8, 9) and, to a lesser extent, in the periphery (10). In adoptive transfer experiments we have shown that diabetes in NOD mice can be prevented by the introduction of exogenous DN thymic NKT cells and that this protection is mediated by IL-4 (11). Lehuen et al. (12) subsequently confirmed the protective role of NKT cells in NOD mice by increasing the numbers of these cells by introducing a transgene encoding the NKT cell–associated TCR Vα14Jα281 chain. This association between a deficiency in NKT cells and autoimmune diabetes also holds true for other species. Diabetes-prone BioBreeding (BB) rats have approximately one-tenth the proportion of $NKR\text{-}P1^+TCR^+$ lymphocytes in their spleens and livers compared with that of diabetes-resistant BB rats (13), and human diabetic individuals have been found to have lower frequencies of DN Vα24JαQ T cells in peripheral blood than do their nondiabetic siblings (14). Similarly, the onset of systemic lupus erythematosus in lupus-prone mouse strains also correlates with a depletion of $NK1.1^+$ NKT cells (15, 16).

Unfortunately, NKT cells cannot be viewed simply as immune suppressor cells, since they also play a proinflammatory role in host resistance to tumors and infection. Probably the best-established role for NKT cells is in immune responses to mycobacterial lipid antigens. For over a decade, Porcelli and Brenner have studied a population of human DN TCR^+ T cells that are restricted by CD1 and respond to mycobacterial lipid-containing antigens such as mycolic acid and lipoglycan lipoarabinomannan (17–20). The exact relationship of these cells to $V\alpha24^+$ NKT cells in humans or $NKR\text{-}P1^+$ NKT cells in rodents is not clear, since they are not restricted by CD1d, but by the highly homologous molecules CD1a or CD1b (17, 18, 20). Despite this complication, the only member of the CD1 family of molecules expressed in mice is CD1d, and the NKT cells present in mice also respond to mycobacterial antigens. For example, they predominate in the granulomatous reaction to *Mycobacterium tuberculosis* cell wall preparations, and such granulomas do not form in NKT cell–deficient Jα281 TCR–targeted deletion mice (21). The NKT cells of normal mice respond to mycobacterial infection by decreasing IL-4 production and increasing IFN-γ production (22), changes highly likely to aid the host response to mycobacteria, since IFN-γ plays a critical role in pathogen clearance (23, 24).

> Because it is believed that NKT cells recognize a restricted range of targets, it is not surprising that NKT cells are substantially decreased in mice with targeted deletion of CD1d.

NKT cells contribute to the immune response against *Listeria monocytogenes* via a mechanism mediated by monocyte chemoattractant protein-1 (MCP-1) (25, 26). In an experimental model of malaria, they inhibit infection with *Plasmodium yoelii* sporozoites via an IFN-γ–dependent mechanism (27). In antitumor responses, NKT cells usually act via IL-12–dependent (28, 29), perforin-mediated cytotoxicity (29, 30), although IL-4 production by NKT cells also appears to play a role in the induction of massive tumor necrosis in at least one model (31).

Ikehara et al. (1) have now identified a role for NKT cells in mediating the transplantation tolerance induced by anti-CD4 mAb therapy. In their model, the survival of rat islet xenografts transplanted to the livers of mice appeared to depend on preferential loss of conventional $CD4^+$ T cells, rather than $CD4^{int}$ NKT cells, upon treatment with an mAb to CD4. A dose of this mAb that depleted the former, but not the latter, cell population enhanced graft survival, whereas a higher dose, which resulted in prolonged depletion of both cell subsets, did not. It is unclear whether this therapy led to the death of CD4-bearing cells or to the loss of

this marker from their surface.

When the authors examined the efficacy of their anti-CD4 mAb therapy in mice with a targeted deletion of the Vα14 TCR chain, they found that they were no longer able to induce tolerance (1). As these mice are unable to generate the Vα14Jα281 TCR chain, they were severely deficient in NKT cells. The finding that the efficacy of the treatment was restored when NKT-cell numbers were reconstituted with cells from Vα14 TCR transgenic mice supported the hypothesis that NKT cells mediated tolerance induction in this model.

As IL-4 and IFN-γ play important roles in the regulatory activities of NKT cells, Ikehara et al. attempted to study the involvement of these cytokines by repeating their experiments in mice carrying targeted deletions of the gene for either cytokine. Surprisingly, low-dose anti-CD4 mAb was effective in IL-4–deficient mice, indicating that IL-4 production was not essential for the anti-inflammatory activity of NKT cells. The role of IFN-γ could not be determined, since rejection of rat islets was dependent on this cytokine and did not occur, even in the control mice which did not receive NKT cells.

The flexibility of NKT-cell responses

NKT-cell involvement in this and other models seems to show that these cells play adaptive roles in almost every type of immune response. They protect transplants, fight infections, kill tumors, and prevent autoimmune diseases. NKT cells seem to be able to triage a wide range of antigenic challenges and respond appropriately. The problem is that it is difficult to envisage how NKT cells, which express minimally varying TCRs, can differentiate one immunological challenge from another. The belief that NKT cells are activated through a relatively invariant TCR seems inconsistent with their apparent sensitivity and flexibility in a range of situations.

There are at least three possible explanations for the apparent omniscience of these cells. The first is that, despite the appearances, the responsibility of deciding the nature of the immune response does not lie with the NKT cell itself. Rather, the NKT cell may act as an impartial amplifier of immune responses that have their characters determined by other cell types, such as antigen-presenting cells or conventional $CD4^+$ T cells.

The second possibility is that the signal inputs available to the NKT cell are more numerous and varied than they appear. For example, NKT cells are clearly responsive to a number of cytokines, including IL-12 and IL-15. They are also likely to express a substantial number of NK cell–associated surface receptors in addition to those that have already been identified. As these receptor families are very extensive, and as poorly characterized as they are variable, there is a great deal of scope for fine control of effector function.

The third explanation is that NKT cells may not be as homogenous as they seem. It is possible that different subsets are activated under different conditions and respond in different ways. There are obvious divisions between the $CD4^+$ and DN NKT cells, as well as potential divisions between those that express different TCRβ chains (32). It is also possible that the NKT cell–like cells that do not express NKR-P1, or do not appear to rely on antigen presentation by CD1, make important contributions to the nature of some immune responses. The most obvious range for inquiry into NKT cell specialization lies in the human, where NKT cells potentially can respond to one of four different CD1 molecules, each of which could be responsible for presenting different nonpeptide antigens under varying conditions.

Acknowledgments

The author would like to thank Stuart Tangye and Lynn Poulton for their critical reading of the manuscript. Alan G. Baxter is an R.D. Wright Fellow of the Australian National Health and Medical Research Council (NHMRC) and is funded by the NHMRC and the Juvenile Diabetes Foundation.

Alan G. Baxter is the author of *Germ warfare: breakthroughs in immunology*. 2000. Allen and Unwin. Sydney, Australia.

1. Ikehara, Y., et al. 2000. $CD4^+$ Vα14 natural killer T cells are essential for acceptance of rat islet xenografts in mice. *J. Clin. Invest.* **105**:1761–1767.
2. Zlotnik, A., Godfrey, D.I., Fischer, M., and Suda, T. 1992. Cytokine production by mature and immature CD4-CD8- T cells. αβ-T cell receptor+ CD4-CD8- T cells produce IL-4. *J. Immunol.* **149**:1211–1215.
3. Yoshimoto, T., Bendelac, A., Watson, C., Hu-Li, J., and Paul, W.E. 1995. Role of NK1.1+ T cells in a Th2 response and in immunoglobulin E production. *Science.* **270**:1845–1847.
4. Porcelli, S., Yockey, C.E., Brenner, M.B., and Balk, S.P. 1993. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- α/β T cells demonstrates preferential use of several Vβ genes and an invariant TCR α chain. *J. Exp. Med.* **178**:1–16.
5. Lantz, O., and Bendelac, A. 1994. An invariant T cell receptor α chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4-CD8- T cells in mice and humans. *J. Exp. Med.* **180**:1097–1106.
6. Chen, Y.-H., Chiu, N.M., Mandal, M., Wang, N., and Wang, C.-R. 1997. Impaired NK1+ T cell development and early IL-4 production in CD1-deficient mice. *Immunity.* **6**:459–467.
7. Mendiratta, S.K., et al. 1997. CD1d1 mutant mice are deficient in natural T cells that promptly produce IL-4. *Immunity.* **6**:469–477.
8. Gombert, J.-M., et al. 1996. Early quantitative and functional deficiency of NK1+-like thymocytes in the NOD mouse. *Eur. J. Immunol.* **26**:2989–2998.
9. Godfrey, D.I., Kinder, S.J., Silveira, P., and Baxter, A.G. 1997. Flow cytometric study of T cell development in NOD mice reveals a deficiency in αβTCR+CD4-CD8- thymocytes. *J. Autoimmun.* **10**:279–285.
10. Baxter, A.G., Kinder, S.J., Hammond, K.J.L., Scollay, R., and Godfrey, D.I. 1997. Association between αβTCR+ CD4-CD8- T-cell deficiency and IDDM in NOD mice. *Diabetes.* **46**:572–582.
11. Hammond, K.J.L., et al. 1998. Alpha/beta-T cell receptor (TCR)+CD4-CD8- NKT thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic NOD/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. *J. Exp. Med.* **187**:1047–1056.
12. Lehuen, A., et al. 1998. Overexpression of natural killer T cells protects V-alpha-14-J-alpha-281 transgenic nonobese diabetic mice against diabetes. *J. Exp. Med.* **188**:1831–1839.
13. Iwakoshi, N.N., Greiner, D.L., Rossini, A.A., and Mordes, J.P. 1999. Diabetes prone BB rats are severely deficient in natural killer T cells. *Autoimmunity.* **31**:1–14.
14. Wilson, S.B., et al. 1998. Extreme Th1 bias of invariant Vα24JαQ T cells in type 1 diabetes [erratum 1999, **399**:84]. *Nature.* **391**:177–181.
15. Takeda, K., and Dennert, G. 1993. The development of autoimmunity in C57BL/6 lpr mice correlates with the disappearance of natural killer type 1-positive cells: evidence for their suppressive action on bone marrow stem cell proliferation, B cell immunoglobulin secretion, and autoimmune symptoms. *J. Exp. Med.* **177**:155–164.
16. Mieza, M.A., et al. 1996. Selective reduction in Vα14+ NKT cells associated with disease development in autoimmune-prone mice. *J. Immunol.* **156**:4035–4040.
17. Porcelli, S., et al. 1989. Recognition of cluster of differentiation 1 antigens by human CD4-CD8- cytolytic T lymphocytes. *Nature.* **341**:447–450.
18. Porcelli, S., Morita, C.T., and Brenner, M.B. 1992. CD1b restricts the response of human CD4-8- T lymphocytes to a microbial antigen. *Nature.* **360**:593–597.
19. Beckman, E.M., et al. 1994. Recognition of a lipid antigen by CD1-restricted αβ+ T cells. *Nature.* **372**:691–694.
20. Sieling, P.A., et al. 1995. CD1-restricted T cell recognition of microbial lipoglycan antigens. *Science.* **269**:227–230.
21. Apostolou, I., et al. 1999. Murine natural killer (*sic*) cells contribute to the granulomatous reaction caused by mycobacterial cell walls. *Proc. Natl. Acad. Sci. USA.* **96**:5141–5146.
22. Emoto, M., Emoto, Y., Buchwalow, I.B., and Kaufmann, S.H.E. 1999. Induction of IFN-γ producing CD4+ natural killer T cells by

Mycobacterium bovis bacillus Calmette-Guerin. *Eur. J. Immunol.* **29**:650–659.
23. Cooper, A.M., et al. 1993. Disseminated tuberculosis in IFN-γ gene disrupted mice. *J. Exp. Med.* **178**:2243–2247.
24. Flynn, J.L., et al. 1993. An essential role for IFN-γ in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med.* **178**:2249–2254.
25. Flesch, I.E.A., Wandersee, A., and Kaufmann, S.H.E. 1997. IL-4 secretion by CD4+ NK1+ T cells induces monocyte chemoattractant protein-1 in early Listeriosis. *J. Immunol.* **159**:7–10.
26. Kadena, T., et al. 1997. TCRαβ+ CD4- CD8- T cells differentiate extrathymically in an Lck-independent manner and participate in early response against *Listeria monocytogenese* infection through interferon-γ production. *Immunology.* **91**:511–519.
27. Pied, S., et al. 2000. Liver CD4-CD8- NK1.1+ TCRαβ intermediate cells increase during experimental malaria infection and are able to exhibit inhibitory activity against the parasite stage in vitro. *J. Immunol.* **164**:1463–1469.
28. Seki, S., et al. 1997. Antimetastatic effect of NK1+ T cells on experimental haematogenous tumour metastases in the liver and lungs of mice. *Immunology.* **92**:561–566.
29. Smyth, M.J., et al. 2000. Differential tumour surveillance by natural killer (NK) and NKT cells. *J. Exp. Med.* **191**:661–668.
30. Nicol, A., et al. 2000. Human invariant Vα24+ natural killer T cells activated by α-galactosylceramide (KRN7000) have cytotoxic antitumour activity through mechanisms distinct from T cells and natural killer cells. *Immunology.* **99**:229–234.
31. Nakamura, E., et al. 1997. Involvement of NK1+CD4-CD8- αβ T cells and endogenous IL-4 in non-MHC-restricted rejection of embryonal carcinoma in genetically resistant mice. *J. Immunol.* **158**:5338–5348.
32. Hammond, K.J.L., et al. 1999. NKT cells are phenotypically and functionally diverse. *Eur. J. Immunol.* **29**:3768–3781.