Commentary

Immune Privilege: Keeping an Eye on Natural Killer T Cells

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The concept of immune privilege refers to the observation that tissue grafts placed in certain anatomical sites, including the brain and eye, can survive for extended periods of time (1). Immune privilege is thought to reflect an evolutionary adaptation to protect vital structures from damage by inflammatory responses directed against pathogens. It was originally believed that antigens in immune-privileged sites are concealed from the immune system by physical barriers and therefore ignored. However, subsequent studies have shown that antigens do leave immune-privileged sites, that these antigens can induce immune responses, and that immune effector cells can have access to immune-privileged sites. It is now clear that immune privilege is maintained by an active rather than passive process (2–4).

The eye is an excellent example of an immune-privileged site. This organ enjoys immune privilege to protect it from destructive inflammation that may impair vision. Immune privilege in the eye is attributed to a variety of mechanisms (for reviews, see references 2–4), including lack of lymphatic drainage, low expression of MHC molecules, increased expression of surface molecules (CD59, membrane cofactor protein, and decay-accelerating factor) that inhibit complement activation, local production of immunosuppressive cytokines such as TGF-β, presence of neuropeptides, and constitutive expression of Fas ligand (FasL). The latter mechanism controls the entry of Fas-expressing lymphoid cells into the eye (5). Additionally, antigen presentation in the eye can elicit regulatory T (Tr) cells that induce immune deviation and suppress inflammatory responses, both locally and systemically (for a review, see reference 2). In this issue, Sonoda et al. (6) demonstrate that immune deviation induced by antigens placed in the anterior chamber (AC) of the eye requires CD1d-reactive natural killer T (NKT) cells. These findings provide insights into the maintenance of immune privilege and indicate that NKT cells play a critical role in the regulation of immune responses.

AC-associated Immune Deviation

It is well established that injection of antigens into the AC of the eye results in a form of immune deviation termed AC-associated immune deviation (ACAID; for a review, see reference 2). This phenomenon was first identified by Kaplan and Streilein (7), who reported that injection of F1 lymphoid cells into the AC of rat eyes alters the recipient's systemic immune response such that rejection of subsequent allografts from the donor strain used for AC priming is impaired. Since then, ACAID has been demonstrated for several antigens, including viruses, corneal allografts, and tumor cells. The hallmarks of ACAID are suppression of delayed-type hypersensitivity (DTH) and inhibition of the synthesis of complement-fixing antibody isotypes.

Antigen introduced into the AC is captured by specialized APCs of the eye that carry the antigen via the blood to the spleen. A deviant form of immunity is then generated which is capable of suppressing Th1-mediated DTH responses and production of complement-fixing antibodies. $CD8^+$ T cells isolated from the spleen of AC-immunized mice can induce ACAID and inhibit DTH responses when transferred into naive syngeneic mice (8). These findings strongly indicate that AC immunization results in the induction of $CD8^+$ Tr cells that inhibit Th1 cells (see Fig. 1). Interestingly, since ACAID in this system is induced by soluble antigen, presentation of antigen to $CD8^+$ Tr cells must follow the poorly characterized exogenous pathway of MHC class I–restricted antigen presentation (4).

There is considerable evidence that the immunosuppressive cytokine TGF-β plays a critical role in the induction and/or effector function of $CD8^+$ Tr cells during ACAID (2). For example, AC injection of antigen induces the generation of T cells in the spleen that produce TGF-β when stimulated with antigen in vitro. Studies by Griffith et al. (9) further indicated that immune tolerance after antigen injection in the eye depends on the death of lymphoid cells by the Fas/FasL system. Although the exact mechanism by which apoptosis of lymphoid cells affects ACAID remains unclear, it was suggested that apoptotic lymphoid cells produce IL-10 which, in turn, induces immune deviation by its effects on APCs (10). The paper from Sonoda et al. (6) provides another piece to this puzzle, demonstrating that NKT cells are required for the induction of $CD8^+$ Tr cells.

NKT Cells

NKT cells represent a relatively novel lymphocyte subset distinct from conventional T cells, B cells, and NK cells. NKT cells share receptor structures with both NK cells and conventional T cells (for reviews, see references 11, 12). NKT cells express typical NK cell markers, including IL-2Rβ, members of the NKR-P1 NK cell–activating receptor family, and members of the Ly49 NK cell–inhibitory receptor family. They also express a TCR with an invariant Vα14-Jα281 chain and a polyclonal Vβ8.2 (and to a lower extent, Vβ7 or Vβ2) chain. Approximately 60% of all NKT cells

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express CD4, and the remaining cells do not express either CD4 or CD8. These cells have a memory phenotype, expressing high levels of the early activation marker CD69, high levels of CD44 (Pgp-1), and low levels of CD62L (L-selectin). NKT cells are abundant in the thymus and represent a major population of lymphocytes in the spleen, bone marrow, and liver, but are rare in lymph nodes.

Unlike conventional T cells that recognize peptide antigens in the context of self-MHC class I or class II molecules, NKT cells are specific for glycolipid antigens bound with the MHC class I–like molecule, CD1d (for reviews, see references 11, 12). As CD1d expression is required for the development of NKT cells, CD1d-deficient mice have a marked reduction in the number of NKT cells (13–15). Autoreactivity appears to be common among NKT cells. Many NKT cell hybridomas were able to react with splenocytes, thymocytes, or CD1d-transfected cells (11). Interestingly, individual hybridomas differed in their reactivity with CD1d-expressing cells, suggesting substantial heterogeneity among NKT cells (16, 17). Recent studies have shown that CD1d can bind with a variety of glycolipids, including phosphatidylinositol derivatives (18, 19) and glycosylceramides (20). However, the physiological significance of these glycolipids for NKT cell function is unclear.

When activated through their TCR, NKT cells become cytotoxic and quickly produce a variety of cytokines, including large amounts of IL-4, significant amounts of IFN-γ, and some inhibitory cytokines such as TGF-β and IL-10 (11, 21). Although their physiological functions remain unclear, NKT cells have been implicated in immune responses against infectious agents, bone marrow grafts, tumors, and self-antigens (11, 12).

Role of NKT Cells in the Development of ACAID

The paper by Sonoda et al. (6) provides convincing evidence that NKT cells are required for development of ACAID. AC administration of antigen to NKT cell–deficient CD1d knockout mice did not induce ACAID, unless these animals were reconstituted with NKT cells from wild-type mice together with CD1d-expressing APCs. Since anti-CD1d antibodies inhibited development of ACAID in wild-type animals, direct interaction of the invariant Vα14 TCR with CD1d appears to be required. The question then arises as to what NKT cells recognize in the spleen after AC administration of protein antigen. One possibility is that eye APCs express, as a result of exposure to immunosuppressive cytokines such as TGF-β, high levels of CD1d that activate autoreactive NKT cells. Alternatively, introduction of antigen into the AC may cause expression of specific endogenous glycolipids that activate NKT cells.

At what step do NKT cells contribute to the induction of ACAID? Prior studies have shown that $CD8^+$ T cells from the spleen of AC-primed mice can adoptively transfer ACAID to naive animals (8). The studies of Sonoda et al. (6) further extend these findings by demonstrating that NKT cell–depleted spleen cell populations from AC-primed animals can inhibit DTH reactions after adoptive transfer to naive animals. Therefore, these findings indicate that NKT cells are specifically required for the generation of antigen-specific $CD8^+$ Tr cells.

A likely scenario for the induction of ACAID, based on the findings of Sonoda et al. (6), is presented in Fig. 1. Antigen inoculated into the AC of the eye is captured by specialized APCs that carry the antigen to the spleen. These

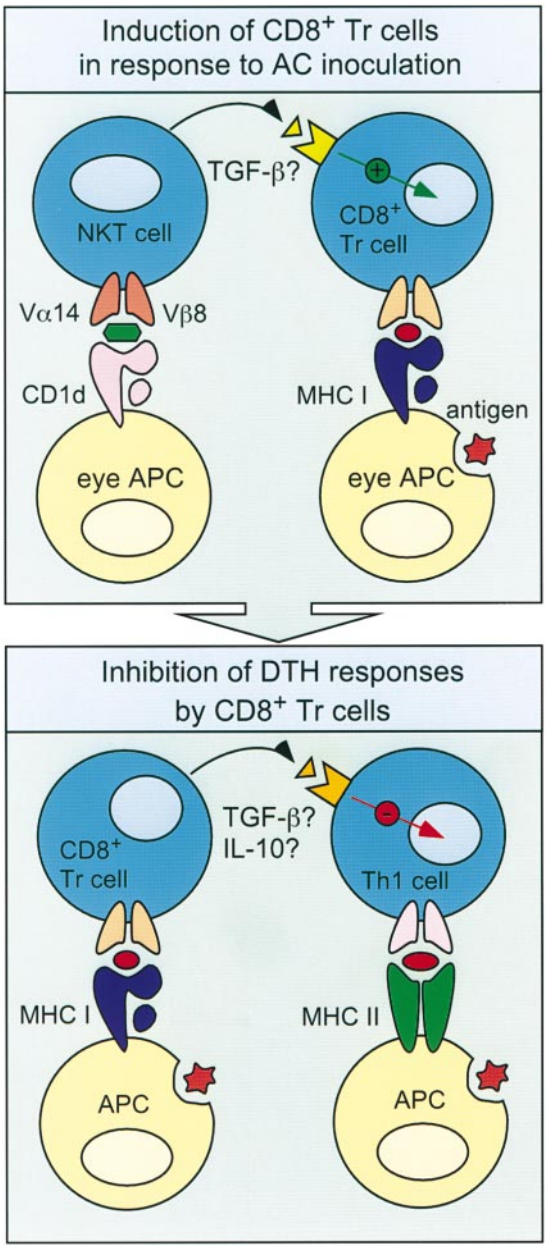

Figure 1. Model for the role of NKT cells in the induction of immune deviation after inoculation of antigen into the eye. Antigen injected into the eye is captured by eye APCs that carry the antigen to the spleen. These eye APCs activate NKT cells which, in turn, produce cytokines such as TGF-β that induce the generation and expansion of antigen-specific MHC class I–restricted $CD8^+$ Tr cells. These $CD8^+$ Tr cells, by production of cytokines such as TGF-β and IL-10, can downregulate subsequent Th1-mediated DTH reactions against the same antigen. Note that presentation of antigen to $CD8^+$ Tr cells in this system follows the exogenous pathway of MHC class I–restricted antigen presentation, which is poorly understood.

eye APCs may have unique features that are critically important for the induction of ACAID, including increased expression of CD1d (perhaps induced by TGF-β in the AC) and altered antigen-presenting properties (perhaps in response to IL-10 produced by apoptotic lymphoid cells). These eye APCs then activate effector T cells, $CD8^+$ Tr cells, and NKT cells. Activated NKT cells produce cytokines (TGF-β is a likely candidate) that stimulate the generation and expansion of antigen-specific $CD8^+$ Tr cells. In turn, these $CD8^+$ Tr cells can inhibit, by production of cytokines (TGF-β and IL-10 are likely candidates), subsequent Th1-mediated DTH responses to the same antigen.

Implications

There is now compelling evidence that Tr cells participate in many immune responses, including responses against foreign and self-antigens (22). The findings of Sonoda et al. (6) raise the possibility that activation of NKT cells is a general mechanism for the generation of Tr cells. Prior studies have suggested a role for NKT cells in the regulation of immune responses. Various mouse strains with genetic susceptibility for the development of autoimmune disease, including nonobese diabetic (NOD) mice with a propensity for development of type I diabetes, SJL mice with a susceptibility for development of experimental allergic encephalomyelitis, and several lupus-prone strains, were found to have defects in NKT cell development and/or function (23–26). Reconstitution of NOD mice with NKT cells (27) or transgenic overexpression of NKT cells in these animals (28) was able to inhibit development of diabetes. Further, NKT cells isolated from Vα14-Jα281 transgenic mice either induced or prevented murine lupus in an adoptive transfer model (29), depending on the source and cytokine profiles of the NKT cells that were transferred. Defects in NKT cell development and function were also observed in human patients with systemic sclerosis (30) and in patients with type I diabetes (31). In addition to their role in the regulation of autoimmunity, NKT cells were shown to influence immune responses against tumors (21) and infectious agents (19, 32–34), and to suppress graft versus host disease (35). In many of these studies it was suggested that NKT cells influence the disease process by production of cytokines. For example, the inhibitory role of NKT cells for the development of Th1-dominated inflammation in several autoimmune diseases was attributed to their production of IL-4, which promotes Th2 immunity. An alternative explanation, not excluded by these studies and supported by the findings of Sonoda et al. (6), is that NKT cells induce the generation of Tr cells that, in turn, inhibit inflammation.

It is unlikely that NKT cells participate in all types of immune regulation controlled by Tr cells. Indeed, Sonoda et al. (6) showed that NKT cells are dispensable for the systemic tolerance induced in response to intravenous injection of antigen. Perhaps other cell types, such as γ/δ T cells and NK cells, can contribute to the regulation of immune responses and induction of self-tolerance. A role for γ/δ T cells as regulators of immune responses has been suggested (for a review, see reference 22).

The realization that NKT cells play a critical role in immune regulation and maintenance of self-tolerance raises the possibility of manipulating these cells to modulate immune responses during prophylaxis and therapy. This should be possible with reagents such as the glycolipid α-galactosylceramide that selectively activates NKT cells (20). Recent studies have shown that administration of this agent to mice can polarize adaptive immune responses towards Th2-dominated immunity (36, 37).

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