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NK Cells Regulate CD4 Responses Prior to Antigen Encounter

Kennichi C. Dowdell,* Daniel J. Cua,† Erlinda Kirkman,‡ and Stephen A. Stohlman*†

NK cells not only respond rapidly to infection, shaping subsequent adaptive immunity, but also play a role in regulating autoimmune disease. The ability of NK cells to influence adaptive immunity before Ag exposure was examined in a gender-dependent model of preferential Th1 and Th2 activation. The inability of young adult male SJL mice to activate Th1 cells was reversed via depletion of NK1.1+ cells, whereas the presence or the absence of NK1.1+ cells did not alter responses in age-matched females. Consistent with a gender-dependent role in regulating adaptive immunity, significantly more NK1.1+ cells were present in males compared with females, and this difference was reversed by castration. In contrast to NK1.1+ cells derived from C57BL/6 mice, no spontaneous cytokine secretion was detected in NK1.1+ cells derived from either male or female SJL mice, although an increased frequency of IL-10-secreting NK1.1+ cells was observed in males vs females following in vitro stimulation. Direct evidence that NK1.1+ cells in males influence CD4+ T cell activation before Ag exposure was demonstrated via the adoptive transfer of APC from control and NK1.1-depleted males. The absence of a functional NK T cell population in SJL mice suggests that NK cells influence adaptive immunity before Ag exposure via alterations in APC activity. The Journal of Immunology, 2003, 171: 234–239.

Natural killer cells are critical components of the innate immune system that respond rapidly to a variety of insults via cytokine secretion and cytolytic activity. Activation results in the secretion of multiple cytokines that play critical roles in modulating adaptive immune responses (1–6). Inbred strains of mice are classified based on NK cytolytic activity: high responder strains exhibit spontaneous cytolytic activity that is enhanced by IFN; intermediate responder strains, e.g., C57BL/6 and BALB/c, exhibit basal cytolytic activity that is readily increased by IFN; and low responder SJL mice exhibit little spontaneous cytolytic activity, which is very poorly augmented by IFN (7).

NK cells have been implicated in the regulation of a variety of both animal models and human autoimmune diseases, including lupus, diabetes, and multiple sclerosis. NK cell depletion worsens murine lupus, while adoptive transfer delays disease onset (8), suggesting that NK cells play a regulatory role. In the murine model, the severity of experimental autoimmune encephalomyelitis (EAE) is increased by NK cell depletion (9). During EAE, NK cells inhibit Ag- and cytokine-induced CD4+ T cell proliferation independent of CD8− or NK1.1− T cells (10). In support of a role for NK cells in autoimmune disease of the CNS, NK cells derived from multiple sclerosis patients express elevated IL-5 and IL-12Rβ2 during remission. During relapses, these NK cells lose their Th2 phenotypic properties (11), suggesting a role in regulating clinical disease.

SJL mice, which are sensitive to both active and passive EAE, are low NK responders due to a unique defect in thymic processing (7). This phenotype is altered to inducible by thymectomy, but not in chimeras prepared with bone marrow cells derived from either inducible or high responder phenotype donors (7). Consistent with a defect in thymic processing, reconstitution of irradiated high phenotype recipients with bone marrow cells derived from SJL donors results in an NK population exhibiting an inducible cytolytic phenotype. SJL mice have limited numbers of CD3− NK1.1− cells and fewer CD3+ NK1.1+ T cells, although a slightly larger population of NK T cells has been identified based on CD1dα-galactosceramide tetramer staining (12) or CD122 and TCR coexpression (13). In contrast to NK T cells in other strains of mice, NK T cells in SJL mice are unable to secrete IL-4 or IFN-γ following TCR ligation (14). Consistent with the inability to secrete IL-4, a unique IL-4 genetic defect in NK T cells derived from SJL mice has been described (13), independent of NKR-P1 gene expression. Together these data indicate that SJL mice not only have a reduced frequency of NK1.1+ T cells relative to other strains of mice, but these cells also appear to lack the functional capabilities ascribed to NK T cells. Furthermore, in many, but not all, strains of mice, NK and NK T cells express a surface Ag recognized by anti-NK1.1 mAb, encoded by the NKR-P1C gene, which transduces an activation signal (13). Recently it has been suggested that the unique low NK responder phenotype in SJL mice is due to the expression of the NKR-P1B gene product, also recognized by anti-NK1.1 mAb, which transduces a negative signal (15).

In addition to a paucity of NK cells (13, 16), CD5+ B cells (17), CD4+CD25+ T regulatory cells (18), a limited Vβ repertoire (19), and defective NK T cells (13, 14), SJL mice exhibit a gender-dependent preferential activation of Th1/Th2 cells (20). Differential responsiveness, resulting in an Ag-specific Th1 response in females and castrated males vs a Th2 response in males (21, 22), is controlled before Ag encounter by a subset of macrophages that function as APC (21, 23, 24). APC function in young adult SJL males is regulated by both IL-4 and IL-10 before Ag encounter.

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4 Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; DTH, delayed-type hypersensitivity; KLH, keyhole limpet hemocyanin; PEC, peritoneal exudate cells.
and correlates with the inability of male-derived APC to secrete IL-12 (22). These data suggest the possibility that the unique environment in male SJL mice might affect the frequency or function of the limited number of NK1.1⁺ cells.

This study demonstrates an increased frequency of NK cells in EAE-resistant males compared with age-matched, EAE-susceptible females, and that the frequency decreases following castration, coincident with the acquisition of both activation of Th1 cells and EAE susceptibility. Furthermore, Th1 responses are induced via immunization of NK cell-depleted males, but not control mAb-treated males. In contrast to NK1.1⁺ cells derived from C57BL/6 mice, NK1.1⁺ cells derived from neither male nor female SJL spontaneously secrete cytokines. However, following activation, male-derived SJL NK cells show an increased frequency of IL-10 secretion compared with age-matched females. The adoptive transfer of APC from NK-depleted males into naive male recipients before Ag encounter demonstrates that NK cells play a role in maintaining the male Th2 environment via an alteration in APC activity. These data suggest that NK cells in young adult male SJL mice shape adaptive immune responses before Ag encounter, resulting in the preferential APC-dependent induction of Th2 cells.

Materials and Methods

**Mice**

SJL mice of both genders were obtained from The Jackson Laboratory (Bar Harbor, ME), Harlan Sprague-Dawley (Indianapolis, IN), or the National Cancer Institute (Frederick, MD) at 4–6 wk of age. No differences in responses were detected comparing mice from the three suppliers (data not shown). To examine the effects of castration, males were purchased at 4 wk of age, castrated or sham castrated at 5 wk, and used at 6–7 wk of age. All castrations were conducted under anesthesia, followed by analgesic during recovery. Male SJL mice were housed one or two per cage. Male BALB/cByJ mice were purchased from The Jackson Laboratory, and male C57BL/6 mice were purchased from the National Cancer Institute at 6 wk of age. Animals were housed and maintained in the University of Southern California vivarium.

**NK T cell activation**

Male BALB/c, C57BL/6, and SJL mice at 6 wk of age and age-matched female SJL mice were injected i.v. with 5 μg of anti-CD3 (BD Pharmingen, San Diego CA). Splenocytes were removed 90 min after injection, and 5 × 10⁵ cells were cultured in 24-well plates in RPMI 1640 medium supplemented with 2 mM l-glutamine, 25 μg/ml gentamicin, nonessential amino acids, sodium pyruvate, 5 × 10⁻³ M 2-ME, and 10% prescreened FCS at 37°C. Supernatants were removed after 1-h incubation, and the concentrations of IFN-γ and IL-4 were determined by sandwich ELISA as previously described (20).

**Delayed-type hypersensitivity (DTH) response**

Mice were immunized i.p. with 100 μg of keyhole limpet hemocyanin (KLH; Calbiochem, La Jolla, CA) in 0.5 ml of PBS. Five days postimmunization mice were challenged with 150 μg of KLH in 25 μl of PBS in the left hind footpad or with an equal volume of PBS in the right hind footpad. DTH responses were determined 24 h postchallenge in a blinded fashion by measuring the difference between the thickness of the KLH- and PBS-challenged footpads with a Mitutoyo micrometer (VWR Scientific, Corticos, CA). For NK cell depletion, mice received 250 μg of anti-NK1.1 (mAb PK136) or 250 μg of a control anti-β-galactosidase mAb (GL113) in 0.5 ml of PBS i.p. on days −2, 0, and +2 relative to KLH immunization. Depletion of NK cells was confirmed by flow cytometry.

**Flow cytometric analysis**

Splenocytes depleted of RBC were stained with PE-labeled anti-NK1.1 (mAb PK136) and FITC-labeled anti-CD3 (mAb 145-2C11; both from BD Pharmingen) to determine the frequency of NK and NK T cells. For intracellular cytokine staining, splenocytes were cultured in RPMI 1640 medium supplemented with 2 mM l-glutamine, 25 μg/ml gentamicin, nonessential amino acids, sodium pyruvate, 5 × 10⁻³ M 2-ME, and 1% mouse serum for 20 h, with or without 50 ng/ml PMA and 1 μM ionomycin (Sigma-Aldrich, St. Louis, MO). GolgiStop (BD Pharmingen) was added for the final 4 h of culture to inhibit cytokine secretion. Cells were washed and stained with FITC anti-NK1.1 (mAb PK136), fixed and permeabilized for 15 min in Cytoperm/Cytofix, washed in Permwash, and stained with PE-anti-IL-10 (mAb JES5-2A5), PE anti-IL-4 (mAb 11B11), or PE anti-IFN-γ (mAb XMG1.2; all obtained from BD Pharmingen). Samples were analyzed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) using CellQuest Pro software.

**Adoptive transfers**

APC activity was examined using peritoneal exudate cells (PEC) as a source of APC (21, 23, 24). PEC were induced by i.p. injection of 2 ml of thioglycolate broth (Difco, Detroit, MI) and were harvested from the peritoneal cavity by lavage with 7–10 ml of Joklik-modified MEM (Life Technologies, Grand Island, NY) supplemented with 5 U/ml of heparin (Sigma-Aldrich) and 25 mM HEPES buffer, pH 7.2, 72 h after induction. PEC, containing <7% Ly-6G⁺ and >90% CD11b⁺ cells, were transferred i.p at 5 × 10⁵ to naive males on the day of immunization. KLH-specific DTH responses were determined as described above. The effect of NK cells on APC activity was examined with APC derived from mice depleted of NK cells via i.p. injection of 0.5 ml of PBS containing 250 μg of anti-NK1.1 (mAb PK136) on days −7, −4, and 0 relative to PEC induction. Control mice received 0.5 ml of PBS containing 250 μg of anti-β-galactosidase (mAb GL113). Depletion of NK cells was confirmed by flow cytometry.

**Statistical analysis**

Student’s t tests were used to analyze the significance in experiments with only two experimental groups. One-way ANOVA was used in experiments with three or more groups. Differences were considered significant at p < 0.05. Data are expressed as the mean ± SE.

**Results**

**NK cells regulate Th1 activation in male SJL mice**

To determine whether NK cells play a role in the APC-dependent inability of young adult male SJL mice to mount a Th1-mediated CD4⁺ T cell response, Ag-specific Th1 activation, as measured by induction of DTH responses, was compared in NK-deficient and control mAb-treated young adult male SJL mice. Previous data demonstrated a direct correlation between Ag-specific secretion of Th1 cytokines and DTH responses as well as Ag-specific secretion of Th2 cytokines and a lack of measurable DTH responses (20, 25). Flow cytometric analysis of splenic populations on day 6 after primary immunization confirmed the absence of NK cells in anti-NK1.1-treated mice (data not shown). Age-matched female SJL mice responded regardless of the presence or the absence of NK cells (Fig. 1). Consistent with previous data (24, 25), Ag-specific Th1 CD4⁺ cells were not induced in control mAb-treated male SJL mice (Fig. 1). By contrast, young adult male SJL mice depleted of NK cells exhibited Ag-specific DTH responses identical to the responses in female SJL mice (Fig. 1) and castrated male SJL mice (22). Potential effects of cellular debris or Ab-bound...
debris on APC-dependent DTH responses were examined by the transfer of \(2 \times 10^7\) freeze/thawed splenocytes with or without prior absorption of anti-CD8 mAb. Adoptive transfer of cell debris, either with or without adsorbed Ab, to young adult males before immunization did not result in Th1 activation or inhibit Th1 activation in female SJL mice (data not shown). These data suggest a correlation between NK cells in males and the gender-dependent Th1/Th2 responsiveness of SJL mice. The demonstration that NK cell depletion allows the activation of Th1 effectors in males without altering the response in female mice suggests a role for NK cells within this hormone-dependent pathway.

**Gender-dependent frequency of NK cells in SJL mice**

Based on the ability to activate Ag-specific Th1 cells in NK cell-deficient young adult males, the frequencies of NK1.1\(^+\) cells in low responder SJL mice of both genders were compared with those in age-matched DTH responsive male C57BL/6 mice (Fig. 2). Similar to C57BL/6 mice, splenic NK1.1 expression is predominantly restricted to the CD3\(^-\)NK1.1\(^+\) population in SJL mice. However, consistent with previous reports (12, 13), the frequencies of both NK1.1\(^+\) cells and NK1.1\(^-\) T cells are reduced in SJL mice compared with those in NK-inducible C57BL/6 mice (Fig. 2). Comparison of the frequencies of NK1.1\(^+\) cells in age-matched 6-wk-old male and female SJL mice that have identical spleen cell numbers showed that Th1-responsive females had a significant reduction in both NK1.1\(^+\) NK cells and NK1.1\(^-\) CD3\(^+\) NK T cells compared with Th2 responder male SJL mice (Fig. 2, A and B). Although the reduced frequency of NK cells in SJL mice compared with C57BL/6 mice suggested a possible correlation between NK responder phenotype and frequency, age-matched male BALB/c mice (intermediate NK responders) (26) contained \(\sim 3\%\) DX5\(^+\) NK cells (data not shown), similar to the frequency of total NK1.1\(^+\) cells detected in male SJL mice. Similar frequencies were detected in all three strains of mice using the DX5 pan-NK mAb (data not shown). This suggests the absence of a correlation between NK cell frequency and phenotypic designations based on cytolytic activity (26, 27).

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** Gender-dependent increase in NK cells in male SJL mice. Frequency of NK1.1\(^+\) cells in 6-wk-old C57BL/6 and SJL mice, compared with age-matched female SJL mice. A, Representative flow cytometric analysis of NK1.1\(^+\) and CD3\(^+\) splenocytes. The isotype control was negative (data not shown). B, Male SJL mice have reduced NK1.1\(^+\) cells compared with C57BL/6, but a higher frequency compared with age-matched female SJL. Castration reduces NK cell frequency in males. Data are representative of two to four experiments. *, \(p \leq 0.05\) compared with female or castrated male SJL mice.

The gender-dependent inability of males to mount a Th1 CD4\(^+\) T cell response is reversed by castration (21, 22). To determine whether the increased frequency of NK cells in male vs female SJL mice is a consequence of the hormonal environment, the frequency of total NK1.1\(^+\) cells in 6-wk-old castrated or sham-castrated males was compared with that in age-matched females. Sham-castrated males exhibited a frequency of splenic NK cells identical with that in untreated males. Castration reduced the frequency of NK1.1\(^+\) cells in males to approximately the frequency found in female SJL mice (i.e., \(\sim 2\%\); Fig. 2B) without an alteration in splenic cell numbers. These data are consistent with a gender-dependent effect on NK cell frequency.

**FIGURE 3.** Absence of IL-4 or IFN-\(\gamma\) secretion by SJL-derived NK T cells. Male BALB/c, C57BL/6, and SJL mice (MSJL) at 6 wk of age and age-matched female SJL mice (FSJL) were injected i.v. with 5 \(\mu\)g of anti-CD3. Spleens were removed 1.5 h postinjection, and 5 \(\times\) 10\(^6\) splenocytes were cultured in 24-well plates. Supernatants were removed after 1 h at 37°C, and the concentrations of IFN-\(\gamma\) and IL-4 were determined by ELISA. SJL were compared with either C57BL/6 or BALB/c in individual experiments.

**Increased frequency of IL-10-secreting NK cells in male SJL mice**

The Th2-associated cytokines IL-4 and IL-10 influence APC activity in male SJL mice before Ag encounter (30, 31). Although previous data suggested that IL-10 is derived from the APC population (22, 25), the precise source of these cytokines is unknown. NK cells and NK T cells secrete a variety of cytokines, including IL-4 or IFN-\(\gamma\). Increased frequency of IL-10-secreting NK cells in male SJL mice compared with that in untreated males. Castration reduced the frequency of NK1.1\(^+\) cells in males to approximately the frequency found in female SJL mice (i.e., \(\sim 2\%\); Fig. 2B) without an alteration in splenic cell numbers. These data are consistent with a gender-dependent effect on NK cell frequency.

**FIGURE 3.** Absence of IL-4 or IFN-\(\gamma\) secretion by SJL-derived NK T cells. Male BALB/c, C57BL/6, and SJL mice (MSJL) at 6 wk of age and age-matched female SJL mice (FSJL) were injected i.v. with 5 \(\mu\)g of anti-CD3. Spleens were removed 1.5 h postinjection, and 5 \(\times\) 10\(^6\) splenocytes were cultured in 24-well plates. Supernatants were removed after 1 h at 37°C, and the concentrations of IFN-\(\gamma\) and IL-4 were determined by ELISA. SJL were compared with either C57BL/6 or BALB/c in individual experiments.
gender-dependent differential in cytokine secretion. In the absence of stimulation NK1.1+ cells derived from neither male nor female SJL mice secreted detectable IFN-γ, IL-4, or IL-10. Following activation, a small percentage of NK1.1+ cells from both male and female SJL mice secreted IL-4, IL-10, and IFN-γ (Fig. 4, A and B). Although both IL-4 and IL-10 have been implicated in the gender-dependent induction of Th1/Th2 cells in these mice, no difference in the frequency of NK1.1+ cells secreting IL-4 or IFN-γ was detected. By contrast, an increased frequency of NK1.1+ cells derived from 6-wk-old male SJL mice secreted IL-10 compared with age-matched female mice. It is not clear whether the small frequency of cytokine-secreting cells derived from SJL mice represents distinct populations secreting individual cytokines or, alternatively, the secretion of multiple cytokines by individual cells. The latter possibility is supported by analysis of NK1.1+ cells derived from C57BL/6 mice. In contrast to SJL mice, in which no cytokine secretion was detected before activation, >40% of NK1.1+ cells derived from male C57BL/6 secreted all three cytokines, and the frequency increased ~2-fold following stimulation (data not shown). These data demonstrate an increased frequency of IL-10-secreting NK cells in male SJL mice following in vitro stimulation and raise the possibility that IL-10 derived from NK+ cells in males contributes to the inability to activate CD4+ T cells that secrete Th1 type cytokines following protein Ag immunization.

**NK cell depletion alters APC activity**

The absence of induction of CD4+ T cells expressing Th1 cytokines in male SJL mice is regulated via an APC (21, 24). The data suggest that the increased frequency of NK cells in male SJL mice correlates with the inability to activate Th1 effectors. To determine whether the increased frequency of NK cells in males correlated with changes in function, APC were isolated from male SJL mice secreted IL-10 compared with male SJL mice, including the CD3+ subset of NK cells, play a critical role(s) in both the early response to infection and in immune homeostasis (1, 2). The data in this report support the concept that NK cells also play a role in regulating adaptive immunity before Ag encounter. SJL mice exhibit a unique gender-dependent differential response to immunization with protein Ag (23). Preferential activation of Th1 or Th2 cells is controlled via the effects of gonadal hormones (32–34) on APC activity (22). Immunization results in the induction of Ag-specific Th1 cells in young adult females and in the preferential activation of CD4+ T cells expressing a predominantly Th2 cytokine secretion pattern in young adult male SJL mice (20). Preferential activation of Th2 cells in males can be shifted to the preferential activation of Th1 cells by adoptive transfer of APC derived from Th1-responding females (21) or by castration (21, 22, 32). However, the preferential activation of Th1 cells in females cannot be shifted by the transfer of APC derived from young male SJL mice (21). Furthermore, administration of testosterone to female SJL mice increases IL-10 secretion and decreases the severity of EAE (33). Inhibition of IL-4 or IL-10 before immunization in male SJL mice results in Th1 activation (25), demonstrating that these cytokines influence the environment before Ag exposure. Furthermore, the expression of transgenic IL-10 in EAE-susceptible mice renders them EAE resistant (31, 35), similar to the resistance to active EAE induction observed in male SJL mice (20).

Previous data suggested that a macrophage APC was either the direct or indirect target of gonadal hormone activity (22); however,
gonadal hormone receptors have not been identified on macrophages (36). Analysis of the potential role of NK cells in the pathway linking the male gonads to APC activity was conducted based on the observations that NK cells influence APC maturation (37), and APC influence NK cell function (37–40). The demonstration that NK cell depletion allowed the activation of Th1 effectors in males without altering the response in female mice supports a role for NK cells within this hormone-dependent pathway. Although classified as a low responder strain based on cytolytic activity, the frequency of NK1.1+ cells in young adult male SJL mice, which is lower than that in intermediate responder C57BL/6 mice, is comparable to intermediate responder BALB/c mice. Surprisingly, the data demonstrate that male SJL mice have ~2-fold more NK1.1+ cells compared with age-matched female SJL mice. Castration, which reverses gender-dependent induction of Th2 cells in males, resulting in both activation of Th1 cells and susceptibility to active EAE (21, 22), reduces the frequency of NK1.1+ cells in males to approximately that in females. Although it has previously been suggested that increased levels of the female hormone 17β-estradiol selectively inhibits NK cytotytic function (41), these data suggest that in addition to altering the phenotype of CD4+ T cells (21), gonadal hormones play a role in regulating the frequency of NK1.1+ cells in SJL mice.

NK cells and NK T cells secrete a variety of cytokines, including IL-4 and IL-10 (2, 5, 6). Although SJL mice have a small population of NK1.1+ T cells (12, 13), these data extend previous observations (13, 14) and demonstrate the absence of a link between gender and either IL-4 or IFN-γ secretion by NK T cells in SJL mice. The absence of a functional response by NK T cells in this strain of mice suggests that the phenotype identified is likely to reflect a function of NK1.1+ NK cells in SJL mice. Cytokine secretion was examined to explore the possibility that the NK cells present in males contributed to the IL-4 and/or IL-10 that influence APC activity before Ag encounter (25). In contrast to NK1.1+ cells derived from the intermediate responder C57BL/6 mice, which secreted IFN-γ, IL-4, and IL-10 without stimulation, no spontaneous cytokine secretion was detected from NK cells derived from SJL mice of either gender. The absence of spontaneous cytokine secretion is consistent with the low responder designation based on limited cytolytic potential (27). Following stimulation, a small percentage of NK1.1+ cells derived from male and female SJL mice secreted cytokines. By contrast, stimulation of NK1.1+ cells derived from C57BL/6 mice resulted in the secretion of all three cytokines analyzed by a large percentage of cells. The frequency of NK1.1+ cells derived from C57BL/6 mice secreting cytokines, either spontaneously or following stimulation, exceeded 100%, suggesting that individual NK cells are capable of secreting multiple cytokines. In addition to an increased frequency in male SJL mice, the frequency of NK1.1+ cells secreting IL-10 following activation was increased in males compared with females. The observation that depletion of NK cells from males or inhibition of IL-10 before isolation (25, 30) results in an alteration of APC function suggests a causative relationship. An in vivo environment in which low levels of IL-10 alter APC function without dramatically altering the surface phenotype (30) coupled with the inability to detect NK cell cytokine secretion without stimulation support a potential IL-10-mediated interaction between NK cells and the APC population. However, due to the extremely low frequency of cells secreting IL-10 and the absence of a differential frequency in cells secreting IL-4, it is not possible to rule out a noncytokine, possibly cell-cell, interaction in this model. One possibility in support of a potential cell-cell interaction is recent data demonstrating that the molecule recognized by anti-NK1.1 expressed by SJL mice is encoded by a gene (NKR-P1B) (15) distinct from the gene encoding the NK1.1 epitope in other strains of mice (NKR-P1C) (42). The observation that NKP-P1B encodes a molecule delivering an inhibitory signal suggests the possibility that a gender-dependent increased frequency of NK cells in male SJL mice expressing an inhibitory molecule may modulate the immune response via a cell-cell contact mechanism in contrast to cytokine secretion.

Adoptive transfer of a limited number of APC derived from Th1-responsive females before immunization results in the activation of Th1 cells in males (21, 23, 24), providing a powerful tool to examine the regulation of APC activity. The correlation between gonadal hormones and NK frequency suggested that NK cell frequency and/or activity might correlate with the activation of Th2 cells in male SJL mice. To examine this possibility, NK1.1-depleted males were examined for Th1 activation. In contrast to NK cell-depleted females, which respond normally, Th1 cells were activated in NK-depleted, but not control, males. Furthermore, APC derived from NK cell-depleted males were as efficient as APC derived from females in supporting Th1 activation in naive male recipients in which a Th2 response is normally induced by immunization. These data suggest a correlation between the increased frequency of NK cells in young adult male SJL mice and preferential activation of Th2 cells. Potential effects of cellular debris or activation through FcRs expressed by the APC population (43, 44) were ruled out by transferring splenocyte-derived debris with or without prior absorption of anti-CD8 mAb. Although the present data do not provide an explanation for the interaction(s) of hormones with either the NK cells or the APC that ultimately affects the cytokine profile of Ag-specific CD4+ T cells, the data support the concept that NK cells may play a previously unrecognized role in regulating immune responses before Ag encounter.

References


