NK Cell-Deficient Mice Develop a Th1-Like Response but Fail to Mount an Efficient Antigen-Specific IgG2a Antibody Response

Abhay R. Satoskar, Luisa M. Stamm, Ximing Zhang, Mitsuhiro Okano, John R. David, Cox Terhorst and Baoping Wang

*J Immunol* 1999; 163:5298-5302; [http://www.jimmunol.org/content/163/10/5298](http://www.jimmunol.org/content/163/10/5298)

---

**References**  This article cites 30 articles, 22 of which you can access for free at: [http://www.jimmunol.org/content/163/10/5298.full#ref-list-1](http://www.jimmunol.org/content/163/10/5298.full#ref-list-1)

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions**  Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
NK Cell-Deficient Mice Develop a Th1-Like Response but Fail to Mount an Efficient Antigen-Specific IgG2a Antibody Response

Abhay R. Satoskar, Luisa M. Stamm, Ximing Zhang, Mitsuhiro Okano, John R. David, Cox Terhorst, and Baoping Wang

NK cells have been shown to play a role in the modulation of B cell differentiation and Ab production. Using a novel murine model of NK cell deficiency, we analyzed the in vivo role of NK cells in the regulation of Ag-specific Ab production. After immunization with OVA or keyhole limpet hemocyanin in CFA, NK cell-deficient (NK<sup>−/−</sup>) mice developed an efficient Th1 response and produced significant levels of IFN-γ but displayed markedly reduced or absent Ag-specific IgG2a production. There were no differences in the levels of Ag-specific IgG, IgG1, and IgG2b between NK<sup>−/−</sup> and NK<sup>+/+</sup> mice. Furthermore, NK cell-reconstituted, NK<sup>+/+</sup> (tg26Y) mice produced significant amounts of Ag-specific IgG2a after immunization with OVA. These results indicate that NK cells are involved in the induction of Ag-specific IgG2a production in vivo. Moreover, they also demonstrate that the lack of Ag-specific IgG2a Ab production in NK<sup>−/−</sup> mice is not associated with the impaired Th1 response and IFN-γ production. The Journal of Immunology, 1999, 163: 5298–5302.

Natural killer cells are derived from bone marrow (BM) progenitor cells that express specific surface markers, such as NK1.1 and asialo G<sub>M</sub>1 (ASGM1), and lack B cell- and T cell-specific markers, such as Ig<sub>M</sub>, TCR<sub>α</sub>, CD4<sup>+</sup>, CD8<sup>+</sup>, and CD3γδε<sup>+</sup>. NK cells play an important role in innate immunity against bacterial, parasitic, and viral infections (1). NK cells also exhibit cytotoxicity against tumor cells and neoplasms (1). In addition, NK cells have been shown to regulate hemopoiesis, B cell differentiation, and Ab production (2–13).

Although some studies have reported that NK cells inhibit B cell differentiation and suppress Ab production (10–13), others have demonstrated that NK cells promote B cell growth and increase IgM and IgG Ab production by activated as well as resting B cells (8, 14–16). The role of NK cells in the modulation of B cell response and Ab production has been attributed to their ability to directly interact with B cells and/or produce cytokines that regulate B cell differentiation and isotype switching (15). In fact, NK cell-derived IFN-γ has been shown to induce IgG2a class switching in LPS-activated B cells (15). Although, a majority of these studies demonstrate that NK cells can regulate T cell-independent Ab production from B cells in vitro, it is not clear whether NK cells play a similar role in the regulation of T cell-dependent as well as T cell-independent Ab responses in vivo. T cell-independent Abs such as bacterial polysaccharides cross-link B cell surface Ag receptors and directly activate B cells to produce Abs, whereas the development of Ab responses to T cell-dependent Ags requires help from CD4<sup>+</sup> T cells (16).

Previous studies have demonstrated that NK cell depletion using anti-NK1.1 Ab fails to influence initial CD4<sup>+</sup> T cell commitment, cytokine production (17), or Ab responses in vivo to T cell-dependent and T cell-independent Ags (17, 18). In contrast, the activation of NK cells by poly(I:C) administration increased the levels of both Ag-specific IgG1 and IgG2a isotypes (15). Furthermore, depletion of NK cells using anti-NK1.1 Abs before poly(I:C)-induced activation blocked their ability to enhance IgG2a but not IgG1 production (15). In addition, two independent studies have demonstrated that activated NK cells selectively up-regulate IgG2a production (9, 18). Collectively, these results indicate that activated NK cells, but not endogenous NK cells, play a critical role in the regulation of IgG2a production. Therefore, we analyzed Ag-specific Ab responses and the development of a Th1 response in NK cell-deficient mice immunized with the T cell-dependent Ags OVA or keyhole limpet hemocyanin (KLH) in CFA. Our results indicate that endogenous NK cells are involved in the induction of CFA-induced, Ag-specific IgG2a production in vivo, and this regulation appears to be independent of IFN-γ.

Materials and Methods

Mice

Tg26 mice were maintained through sibling breeding in the animal facility of the Beth Israel Deaconess Medical Center. Mice lacking NK cells (NK<sup>−/−</sup>) were generated by transplanting fetal liver or BM cells from (C57BL/6 × CBA)F<sub>1</sub> mice into neonatal tge26 mice as described recently by us (19). Age- and sex-matched wild-type (wt) CBA/J (H-2<sup>b</sup>) and C57BL/6 (H-2<sup>b</sup>) mice were used. The (C57BL/6 × CBA)F<sub>1</sub> (H-2<sup>b</sup>/wt) mice generated by the breeding of C57BL/6 × CBA/J mice were used as the wt (NK<sup>−/−</sup>) mice. In addition, we also used NK<sup>−/−</sup> (FLwt→tg26W3) mice that were generated by transplanting wt fetal liver cells into 2- to 3-wk-old tge26 mice as described recently (19). NK<sup>−/−</sup> (FLwt→tg26W3) mice were phenotypically and functionally identical with the wt (NK<sup>−/−</sup>) mice and were referred as NK<sup>−/−</sup> (tg26Y). Of note, all of the NK<sup>−/−</sup> mice used in this study were analyzed by flow cytometry of PBLs before immunization; lymph node and spleen cells were
analyzed by flow cytometry after the animals had been sacrificed to confirm the lack of or markedly diminished NK cells and the presence of T cells.

Immunization protocol

OVA (grade V, Sigma, St. Louis, MO) or KLH (Sigma) was emulsified in CFA (Life Technologies, Rockville, MD) by repeated passage through a double-hubbed emulsifying needle until a stable emulsion was formed. Groups of four to five mice were immunized s.c. into shaven back rumps with 0.1 ml of OVA (200 μg) or KLH (200 μg) in PBS, emulsified in CFA. Mice were boosted 2 wk later using OVA (200 μg) or KLH (200 μg) in PBS, emulsified in IFA.

Ab ELISA

Peripheral blood was collected from tail snips of all experimental animals 2 wk after boosting with OVA or KLH in IFA. Blood was centrifuged at 200 × g and serum was collected to determine the titers of Th1-associated IgG2a as well as Th2-associated IgG1 and IgG2b Ag-specific Abs by ELISA as described previously (20).

T cell proliferation and cytokine assays

Spleens were removed from the OVA- or KLH-immunized mice 2 wk after boosting, and T cell proliferation assays were performed as described previously (20). Briefly, single-cell suspensions were prepared by gentle teasing in complete RPMI 1640 medium. The cells were centrifuged at 200 × g for 5 min, and erythrocytes were lysed by resuspending cells in Boyle’s solution (0.17 M Tris and 0.16 M ammonium chloride). A total of 5 × 10⁵ spleen cells were added in triplicate to the wells of 96-well, flat-bottom tissue culture plates and stimulated with either 20 μg/ml or 1 μg/ml of Con A. Culture supernatants from these assays were analyzed for the production of IL-4 (reagents from Endogen, Cambridge, MA; detection limit of 5 pg/ml) and IFN-γ (reagents from PharMingen, San Diego, CA; detection limit of 20 pg/ml) by capture ELISA as described previously (20).

Statistical analyses

Student’s unpaired t test was used to determine the significance of the values obtained. Differences in Ab endpoint titers were determined using the Mann-Whitney U prime test.

Results and Discussion

Immunization of NK<sup>T</sup><sup>+</sup> mice with OVA or KLH fails to induce an efficient Ag-specific IgG2a response

A number of investigators have studied the role of NK cells in the regulation of Ig production in vitro and in vivo (7–15, 21, 22). Although some earlier studies indicated that NK cells can suppress Ig secretion (5, 12, 13), others reported that NK cells can enhance Ig secretion in vitro and in vivo (7–9, 14, 15, 22). These conflicting observations may be attributed to several factors, such as the type of Ag (T cell-independent vs T cell-dependent) and the depletion of NK cells using ASGM1 Abs, which can bind the surface Ag expressed on activated macrophages and T cells (23). Nevertheless, two independent studies in which more specific anti-NK1.1 Ab was used to deplete NK cells found that NK cell depletion failed to modulate in vivo Ab responses against T cell-independent as well as T cell-dependent Ags (15, 17). Similarly, we found that the administration of NK cell-depleting anti-ASGM1 Abs to NK<sup>T</sup><sup>+</sup> mice before immunization with OVA in CFA had a minor effect on IFN-γ production and the OVA-specific IgG2a response (data not shown). The marginally reduced levels of OVA-specific IgG2a observed in some NK-depleted mice correlated with decreased NK activity as assessed by the YAC-1 cell lysis assay (data not shown). Moreover, we found that the degree of NK cell depletion using anti-ASGM1 antisera varies from mouse to mouse and depends upon the day of assay (data not shown). In contrast, poly(I:C)-activated NK cells increased Ag-specific IgG1 and IgG2a levels (15). Furthermore, NK cell depletion by anti-NK1.1 treatment before poly(I:C)-induced activation blocked the enhancement of IgG2a but not IgG1 Ab production (15). These results indicated that although resting, endogenous NK cells do not modulate an in vivo Ab response, poly(I:C)-activated NK cells...
and NK cells are known to possess cytolytic activity and secrete cytokines that can influence adaptive immunity. NK cells are essential for the promotion of an IgG2a response, which is critical for the development of protective immunity against viral infections. NK cells can eliminate virus-infected cells, which can facilitate the induction of protective immunity. This is achieved through the induction of a Th1 response and the secretion of cytokines such as IFN-γ, which helps in the induction of a Th1-like response.

There were no significant differences in the levels of Ig and Ag-specific IgG2b between the groups (data not shown). Taken together, these results indicate that NK cells play a role in the induction rather than in the maintenance of CFA-induced Ag-specific IgG2a responses in vivo. These observations are consistent with our recent study that demonstrated that Leishmania Ag-specific IgG2a production is significantly impaired in L. major-infected NK cell-deficient NK cells (19). The ability of NK cells to preferentially enhance IgG2a production may explain the high titers of virus-specific IgG2a that are observed during viral infections (26). In addition, IgG2a mediates Ab-mediated cytotoxicity (27) and may contribute to the development of protective immunity against virus.

**Impaired Ag-specific IgG2a production in NK cells is not due to lack of Th1 development and IFN-γ production**

Several studies have indicated that the Th1-associated cytokine IFN-γ enhances IgG2a production (28–30). As NK cells are a major source of IFN-γ during the early immune response required for the subsequent development of IFN-γ-producing CD4+ Th1 subset, we compared the IFN-γ production by Ag-stimulated spleen cells from NK cell-deficient mice with that from NK cell-deficient mice. After stimulation with OVA or KLH, spleen cells from NK cell-deficient mice displayed significantly more proliferative responses than did the cells from NK cell-deficient mice. Furthermore, Ag-stimulated splenocytes from both NK cell-deficient mice produced significant and comparable levels of IFN-γ (Fig. 4, A and C). These results indicate that the lack of IgG2a production in NK cell-deficient mice is not due to the impairment of IFN-γ production. Furthermore, there was no difference in the levels of IL-4 between the groups (Fig. 4, B and D).

A previous study demonstrated that injection of BCL1-C11 tumor cells, which induce IFN-γ production by NK cells, specifically enhanced a trinitrophenyl-specific IgG2a response in vivo. The depletion of NK cells using Abs is efficient but transient, as NK cells can be generated from progenitors within a few days. In addition, NK cell-depleting Abs can also deplete subpopulations of T cells and macrophages that also express the same surface Ag (23, 24). The administration of large quantities of anti-NK1.1 or anti-ASGM1 Abs can elicit an immune response to injected Ab in the host. Using a novel model of specific murine NK cell deficiency, we have excluded these possibilities (19). NK cell-deficient mice are truly deficient in NK cell lytic function as determined by the lack cytotoxicity against lymphocytic choriomeningitis virus and YAC-1 cells (19). Furthermore, NK cell-deficient mice have functionally normal CD4+ and CD8+ T cells as assessed by mixed lymphocyte reaction and CTL assays, respectively. CFA has been shown to induce a Th1-like response and Ag-specific IgG1 and IgG2a production (25); therefore, we used CFA as an adjuvant to immunize NK cell-deficient mice and induce production of IgG2a. After immunization and boosting with OVA or KLH in CFA/IFA, NK cell-deficient mice displayed significant titers of Ag-specific IgG1 and IgG2a Abs (Figs. 1, 2, and 3). Moreover, NK cell-deficient mice also produced significant levels of Ag-specific IgG1 and IgG2a Abs after immunization with the Ag in CFA, but before boosting. Interestingly, similarly immunized NK cell-deficient mice also displayed significant titers of Ag-specific IgG1, but had no detectable levels or markedly reduced levels of Ag-specific IgG2a before as well as after boosting with Ag (Figs. 1–3).
CD3ζ−/− mice, which lack functional TCRαβ T cells (9). Moreover, this enhancement of the IgG2a response was IL-12-dependent and was believed to be mediated by NK cell-derived IFN-γ (9). In addition to its ability to induce IFN-γ from NK cells, IL-12 has also been shown to enhance Ab synthesis and IgG2a production to T cell-independent Ags in tgε26 (NK−T−) mice, which lack NK and T cells but still have B cells (31). Nevertheless, we recently demonstrated that despite IL-12 production and Th1 development, NK−T1 mice infected with *L. major* failed to produce *Leishmania*-specific IgG2a (19), indicating that the lack of IL-12 production is an unlikely mechanism responsible for the impairment of IgG2a production in NK−T1 mice.

Reconstitution of the NK cell compartment restores the CFA-induced Ag-specific IgG2a response

We recently found that tgε26 mice reconstituted with (C57BL/6 × CBA/J)F1 (H-2b/k) BM or fetal liver cells at 2–3 wk of age (NK−T− (tgε26Y)) (instead of neonatally as in the generation of NK−T+ mice) resulted in functionally competent NK and T cells that were comparable with those in wt (F1) mice (19). Furthermore, these NK−T− (tgε26Y) mice have a background that is identical with NK−T1 mice. Therefore, we determined whether CFA could induce an Ag-specific IgG2a response in these immunocompetent mice. Unlike NK−T+ mice, NK−T+ (tgε26Y) mice immunized with CFA in OVA produced significant levels of OVA-specific IgG2a (Fig. 5).

In conclusion, NK−T+ mice immunized with the T cell-dependent Ags OVA or KLH in CFA fail to mount an efficient Ag-specific IgG2a response but produce significant total IgG and Ag-specific IgG1 and IgG2b Abs. Furthermore, the inability of NK−T+ mice to mount an efficient IgG2a response is not associated with the lack of Th1 development and IFN-γ production. These results indicate that although NK cells are not involved in the modulation of the Ig response, they play an important role in the selective induction of the CFA-induced IgG2a response to T cell-dependent Ags.

**FIGURE 4.** IFN-γ and IL-4 production by Ag-stimulated splenocytes from NK−T1, NK+T1, and NK−T mice 3 wk after boosting with OVA (A and B) or KLH (C and D). Data are expressed as the mean ± SE. Similar results were observed in two independent experiments for each Ag tested. ND, not detectable.

**FIGURE 5.** OVA-specific IgG1 (A) and IgG2a (B) production in NK−T+ (tgε26Y) and NK−T+ and NK−T− mice 2 wk after boosting with OVA. Data are expressed as reciprocal endpoint titers on a log scale. ND, not detectable.
References