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Natural Killer Cell Depletion Fails to Influence Initial CD4 T Cell Commitment In Vivo in Exogenous Antigen-Stimulated Cytokine and Antibody Responses

MingDong Wang,* Cynthia A. Ellison,*† John G. Gartner,*† and Kent T. HayGlass2*

The role played by NK- and NK1.1-expressing T cells in CD4 T cell activation and induction of immune responses in vivo is controversial. These effector cells of the innate immune response are hypothesized to play a pivotal role in shaping initial T cell activation, with some groups reporting that classical NK cells are required for optimal Th1-like T cell activation, and others supporting a role for NK1.1+ αβ T cells in Th2 generation. Here, we examine the impact of in vivo NK cell depletion on the development of exogenous Ag-specific cytokine and Ab responses using a murine model of human immediate hypersensitivity. OVA-specific immune responses were induced in 1) C57Bl/6 bg/bg and bg/+ mice, 2) BALB/c mice pretreated with anti-asialoGM1 or control Ab, and 3) C57Bl/6 mice depleted of NK1.1-expressing cells by in vivo administration of anti-NK1.1 mAb PK136. Depletion efficacy was assessed by functional assays and flow cytometric analysis. Each of these approaches indicated that depletion of NK cells and NK1.1+CD4+ T cells fails to alter the Th1:Th2 balance of Ag-driven cytokine synthesis, as indicated by OVA-stimulated cytokine synthesis in primary bulk culture. Similarly, the kinetics and intensity of effector responses such as OVA-specific IgG2a and IgE synthesis were neither increased nor decreased in any of the three models examined. The results argue that NK cells and peripheral NK1.1+ T cells do not play an essential role in shaping the induction of Ag-specific immune responses to soluble exogenous Ags, the most common class of inhalant allergen.


E xperimental animal and human clinical research demonstrates a striking relationship among the pattern of lymphokine production induced by Ag exposure, the nature of the resulting immune response, and clinical outcome (1). Although many factors shape the commitment of naïve T cells, the most important appear to be the cytokines present at the time of priming. IL-4 strongly promotes Th2-like activation (3, 4), while IFN-γ, both directly (5, 6) and secondary to IL-12 (7, 8), and likely IL-18, production (9, 10), plays a critical role in promoting Th1-like dominance. Recently, much attention has been devoted to determining the sources of this early cytokine synthesis.

One intriguing hypothesis is that effector cells of the innate immune response play a pivotal role in shaping initial T cell activation (11–14). Activated NK cells exhibit rapid secretion of several cytokines, notably IFN-γ (15). There is compelling evidence that NK cells play a central role in early IFN-γ synthesis in response to infection with a number of protozoal, bacterial, and viral pathogens (16–22). Some superantigens (staphylococcus endotoxin B) have also been demonstrated to directly activate IFN-γ production by NK cells (23).

Collectively, these data have led to a broadly based consensus that early NK cell responses have a profound effect on the characteristics of the ensuing Ag-specific adaptive immune response and are generally required for optimal Th1-like T cell activation (11–14, 20, 24, 25). It should be noted that most of these experimental systems used Ags that tend to be very effective macrophage activators, frequently intracellular pathogens. Several have been shown to be excellent inducers of IL-12 synthesis that stimulate NK cells to promptly produce IFN-γ (7, 8, 26).

At the same time, independent studies implicate NK1.1-expressing T cells, both CD4+CD8− and CD4+CD8+, in providing the initial source of IL-4 for Th2 priming (27–32). These cells express CD3 and an almost invariant αβ TCR specific for the MHC class I-like molecule CD1. They produce high levels of IL-4 and IFN-γ very rapidly upon activation. However, virtually all studies to date have evaluated IL-4/IFN-γ production in vivo or in vitro in response to polyclonal (anti-CD3-, anti-IgD-, or superantigen-mediated) stimulation, rather than physiologic activation by nominal Ag following conventional Ag processing. Indeed, IgE responses were recently reported to be equivalent in CD1-KO and wild-type littermates despite the absence of IL-4-secreting CD1-dependent cells (33). However, Ab and cytokine responses were also exclusively evaluated using anti-IgD and anti-CD3.

Immediate hypersensitivity is the most widespread immunologic disorder in humans. In some countries, allergies represent the most prevalent (and rapidly increasing) chronic health problem among individuals over 15 yr of age (34). The factors responsible for initial induction of Th2-like responses in vivo, and ultimately immediate hypersensitivity, remain incompletely understood. Given that the most widely distributed and clinically prominent class of allergens is the soluble protein Ags, and that the role played by NK cells in steering initial activation of exogenous Ag-specific responses is not known, we investigated the role of NK cells and NK1.1 T cells in the initial induction of such immune...
responses in vivo using three independent approaches. In each, this deficiency was found not to influence the kinetics, intensity, or balance of cytokine (IFN-γ vs IL-4/IL-10 production in primary bulk culture) or Ab (specific IgG2a vs IgE in vivo) responses that developed. The results argue that NK cells and NK1.1+ T cells do not play an essential role in shaping the induction of immune responses to soluble exogenous Ags, the most common class of in-halant allergen.

Materials and Methods

Animals

BALB/c, C57Bl/6, mice, and Sprague-Dawley rats were bred at the University of Maryland, C57Bl/6-Jbg/bg and C57Bl/6-Jbg/+ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were maintained and used in strict accordance with the guidelines issued by the Canadian Council on Animal Care. Mice were seronegative to mycoplasma, Sendai virus, and rodent coronaviruses, including murine hepatitis virus, by ELISA (marine ImmunoComb, Charles River, Lexington, MA).

Treatment of mice and culture conditions

BALB/c were injected i.p. with 40 μl of anti-ASGM1 (Wako Chemicals, Inc., Neuus, Germany) diluted in 0.5 ml of saline, an equivalent amount of normal rabbit IgG, or nothing on days −2 and 0 and every 5 days thereafter until sacrifice. C57Bl/6 were treated with anti-NK1.1 (PK136 ascites at 300 μg mAb/injection) on the same schedule. The optimal amount of Abs for in vivo depletion was determined in preliminary assays of NK activity and flow cytometry (data not shown). For full depletion using anti-NK1.1 this represents approximately sixfold that commonly employed (i.e., 50 μg), a concentration that was recently observed to yield only partial depletion (35). All mice were immunized i.p. with OVA (2 μg in 2 ml of Al(OH)3, adjuvant) or OVA (100 μg) in saline (see Results), then bled for determination of specific Ab production and/or killed for analysis of Ag-driven cytokine production in short term culture. Sera were collected on days 10 and 14, then stored at −20 °C until analyzed for OVA-specific IgG1 and IgG2a levels by ELISA and for IgE levels by passive cutaneous anaphylaxis as previously described (36). For culture, mice were killed 5 days following OVA (alum) immunization or, in experiments conducted in the absence of adjuvants, 8 days after OVA (saline) immunization, times previously found to produce maximal cytokine responses. In time-course experiments, mice were killed between 1 and 8 days postimmunization. Spleen cell suspensions were cultured at 7.5 × 106/ml (2 ml/well) in 24-well plates (Corning Science Products, Rochester, NY) at 37°C in complete medium (37) alone and in the presence of OVA as previously reported. In some experiments, see Results. Mice within each group were bled and cultured individually, with duplicate cultures established for each condition tested. Supernatants were harvested for analysis of IL-4 at 24 h, of IFN-γ at 48 h, and of IL-10 at 96 h. In most experiments, N.K cells were assessed functionally and/or phenotypically at the time of sacrifice.

Analysis of NK cell depletion

NK cell levels were assessed via functional assays and flow cytometry. Briefly, in functional assays YAC-1 and BW1100 target cells were labeled with Na251CrO4 (Amersham, Oakville, Ontario, Canada) at a dose of 50 μCi/1 × 106 cells for 60 min, washed three times in supplemented RPMI 1640, and resuspended to a final concentration of 1 × 106 cells/ml. Ten thousand target cells suspended in 100 μl of medium and various numbers of splenic effector cells, also suspended in 100 μl of medium, were plated into the wells of a plastic 96-well microtiter plate. For each assay performed, four E:T ratios (100 to 12.5:1) were plated in triplicate. The plates were then incubated at 37°C for 4 h in humidified air containing 5% CO2. Following centrifugation at 350 × g, 100 μl of supernatant was harvested from each well and counted for 2 min in a LKB gamma counter (Rockville, MD). The average value (counts per minute) was then determined for each supernatant. The percent lysis was calculated as follows: % lysis = ([CPM (Experimental) − CPM (spontaneous)]/[CPM (maximum) − CPM (spontaneous)]) × 100.

The mean percent lysis and the SE were determined for each triplicate. Dose-response curves were then generated from these values at the specific E:T ratios used in the assay, and lytic units per 106 effector cells were calculated using exponential fit as previously described (38). One lytic unit was defined as the number of effector cells required to achieve 10% cytotoxicity.

Cytokine determinations

IL-4. IL-4 levels were determined using an MTS colorimetric assay employing CT-45 (cells provided by Dr. W. Paul, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) as previously described (39). The specificity of this assay was assured by analysis of representative sample aliquots with anti-IL-4 mAb (11B11), with values inhibited by >85% attributed to IL-4. The presence of rIL-2 did not significantly alter the standard curve obtained with rIL-2. As used here, this assay detects IL-4 at 0.1 to 0.25 U/ml and readily quantitates IL-4 levels above 0.5 U/ml while being unresponsive to IL-2 levels <200 U/ml.

IL-10. A dual mAb-based ELISA employing purified SXC1 and biotinylated, similarly purified SXC2 was used (40). Hybridomas and rIL-10 standard were initially provided by Dr. T. Mosmann (University of Alberta, Edmonton, Alberta, Canada). As described here, this assay detects 0.2 U/ml with quantitation at >0.5 U/ml.

IFN-γ. An ELISA using purified rIFN-γ mAbs XMG 1.2 and purified, biotinylated R4-6A2 (American Type Culture Collection, Rockville, MD) was performed (40). Serial dilutions of IFN-γ-containing, Con A-stimulated spleen cell supernatants, calibrated against World Health Organization-National Institute of Allergy and Infectious Diseases International Reference Reagent Gg02-901-533 (provided by Dr. C. Lauthin, National Institute of Allergy and Infectious Diseases, National Institutes of Health) were included. The lower limit of detection is 0.2 U/ml, with quantitative measurement of IFN-γ ≥0.5 U/ml.

Statistical analysis

Arithmetic mean cytokine, IgG1, and IgG2a production (of two to six experiments, see Results) is shown ± SEM. IgE responses are expressed as geometric means. Statistical significance was determined using unpaired two-tailed Student’s t tests. Statistical significance of differences in mean IFN-γ/IL-4 ratios was determined via a paired Wilcoxon rank sum test.

Results

Development and maintenance of cytokine and OVA-specific Ab responses does not differ in normal and Beige/Beige C57Bl/6 mice

As a complementary approach to evaluating the effectiveness of NK cell depletion, flow cytometric analysis was conducted using a Coulter Electronic EPICS 753 cell sorter with argon ion laser excitation set at 488 nm (500 mW; Coulter Electronics, Hialeah, FL). Forward vs side light scatter histograms were collected to identify and set a bit map gate for single intact lymphocytes; fluorescence histograms were based on 10,000 cells that satisfied this gate. Spleen cells from PK136 (in vivo)-treated and control Ab-treated C57Bl/6 mice were B cell depleted via adherence to anti-IgG2a mAb (MK.D6) followed by biotinylated goat anti-mouse IgG2a (Southern Biotechnology Associates, Birmingham, AL) and streptavidin-phycocerythrin (Pierce, Rockford, IL). The fluorescence signals derived from each cell were split with a 550-nm dichroic longpass filter, with FITC and phycoerythrin signals detected through 525- and 575-nm bandpass filters, respectively. Color compensations were based on test samples stained with the two fluorochromes separately. Data were collected in histogram format and subsequently analyzed using Coulter Elite workstation software, version 4.01.

Development and maintenance of cytokine and OVA-specific Ab responses does not differ in normal and Beige/Beige C57Bl/6 mice

We first examined OVA-specific T cell activation in normal B6, B6 bg/bg, and B6 bg/+ mice. Mice were immunized with OVA and killed 8 days later, the time of peak IFN-γ, IL-4, and IL-10 responses. Examination of these cytokine responses in bulk culture revealed that normal, Beige, and heterozygous control mice exhibited no difference in the intensity of the IFN-γ response, or in the balance between Th1 vs Th2-like activation as indicated by OVA-stimulated IL-4 or IL-10 synthesis in primary culture. The mean ratio of IFN-γ/IL-10 synthesis was virtually identical in these
three strains (1.60, 1.46, and 1.48 in groups I, II, and III, respectively; $p > 0.05$; data calculated from Table I), indicating development of a Th1-like dominance that is of virtually identical intensity in OVA–primed normal B6, B6 bg/bg, and B6 bg/+ mice. Similar ratios were obtained for comparisons of OVA–stimulated IFN-γ and IL-10 synthesis.

As a complementary approach to evaluating the relative balance of Th1-like vs Th2-like activation in these three strains, independent groups of mice were OVA (alum) immunized, and specific Ab production was determined. As demonstrated in Table I, primary and secondary Ag–specific IgE, IgG1, and IgG2a responses did not differ among the three groups.

**Anti-ASGM1 treatment does not detectably alter Th1-like vs Th2-like induction in BALB/c mice**

Mice homozygous for the bg gene are genetically deficient in most classic NK cell functions, and as such have provided a model previously used to support the hypothesis that NK cells play a pivotal role in the initial induction of the Th1-like response. At the same time, the bg/bg defect is not the optimal means of characterizing NK cell function, as it does not abolish all NK cell functions (i.e., IFN-γ synthesis). Consequently, we employed an alternative model of NK depletion: chronic administration of polyclonal anti-asialoGM1 Ab to BALB/c mice. Virtually all murine NK cells express this marker (41), and as widely demonstrated, this approach effectively depletes functional and phenotypic NK cell activity in vivo. Following administration of these Abs, even mice treated with the potent NK cell activator poly(I:C) failed to exhibit demonstrable NK cells. NK cell depletion was effective within 18 h (Table II) and persisted for a minimum of 7 days. Therefore, to ensure NK cell depletion, Ab treatment was given at 5-day intervals, beginning 2 days before OVA immunization and continuing until sacrifice. In some experiments, randomly selected mice within each cohort were killed at various times over the course of the experiments (5–42 days) to confirm that NK cells remained at undetectable levels.

**FIGURE 1.** Anti-asialoGM1 treatment in vivo does not alter induction of Th1- or Th2-like cytokine synthesis patterns. BALB/c mice were treated with anti-ASGM1 or normal rabbit Ig (or in one experiment were left untreated) on days −2 and 0, then OVA (alum) immunized on day 0. Mice were killed on day 3 (not shown) or day 5, and spleen cells were used for primary culture. Cytokine production in the absence of OVA in culture was measured. Data shown are the mean ± SEM of data pooled from three independent experiments, each containing two to four mice cultured and analyzed independently. In the second series of experiments (Ab responses), separate groups of mice were immunized with 2 μg OVA (in alum to elicit Ab production) on days 0 and 28. Mice were bled, and OVA–specific serum Ab production was determined. Data are peak mean ELISA (IgG1, IgG2a) or passive cutaneous anaphylaxis (IgE, geometric means) responses. SEM, omitted for clarity, ranged from 5 to 15% in most instances.

---

**Table I.** Induction and maintenance of OVA-specific cytokine and Ab responses does not differ in B6, B6 bg/+, and B6 bg/bg mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>IFN-γ (U/ml ± SEM)</th>
<th>IL-4 (U/ml ± SEM)</th>
<th>IL-10 (U/ml ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57 B1/6</td>
<td>18.7 ± 2.3</td>
<td>11.7 ± 3.4</td>
<td>16.3 ± 2.9</td>
</tr>
<tr>
<td>B6 bg/+</td>
<td>23.0 ± 3.1</td>
<td>15.7 ± 4.0</td>
<td>25.8 ± 1.7</td>
</tr>
<tr>
<td>B6 bg/bg</td>
<td>24.7 ± 2.9</td>
<td>16.7 ± 2.3</td>
<td>27.9 ± 3.9</td>
</tr>
</tbody>
</table>

$^a$ The indicated strains were immunized with 100 μg OVA (without adjuvants) and sacrificed 8 days later for analysis of cytokine production in short-term primary culture. Data shown are the mean ± SEM of data pooled from three independent experiments, each containing two to four mice cultured and analyzed independently. In the second series of experiments (Ab responses), separate groups of mice were immunized with 2 μg OVA (in alum to elicit Ab production) on days 0 and 28. Mice were bled, and OVA–specific serum Ab production was determined. Data are peak mean ELISA (IgG1, IgG2a) or passive cutaneous anaphylaxis (IgE, geometric means) responses. SEM, omitted for clarity, ranged from 5 to 15% in most instances.

<table>
<thead>
<tr>
<th>Strain</th>
<th>IgE</th>
<th>IgG1</th>
<th>IgG2a</th>
<th>IgE</th>
<th>IgG1</th>
<th>IgG2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVA-Driven Cytokine Response (U/ml ± SEM)</td>
<td>685</td>
<td>40,000</td>
<td>31</td>
<td>1935</td>
<td>867,000</td>
<td>1546</td>
</tr>
<tr>
<td>Primary</td>
<td>761</td>
<td>44,100</td>
<td>41</td>
<td>2440</td>
<td>640,000</td>
<td>1524</td>
</tr>
<tr>
<td>Secondary</td>
<td>949</td>
<td>39,300</td>
<td>33</td>
<td>1824</td>
<td>883,000</td>
<td>1537</td>
</tr>
</tbody>
</table>

**Table II.** Impact of in vivo administration of anti-asialoGM1 on NK cell function: BALB/c

<table>
<thead>
<tr>
<th>Treatment In Vivo</th>
<th>−36 h</th>
<th>−18 h</th>
<th>Specific Cytotoxicity (%) at 100:1, E:T</th>
<th>Lytic Units$_{10^7}$ (per 10$^7$/cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-ASGM1</td>
<td></td>
<td>14</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Anti-ASGM1 polyIC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Anti-ASGM1 polyIC</td>
<td>47</td>
<td>192</td>
<td>192</td>
<td></td>
</tr>
<tr>
<td>Anti-ASGM1 polyIC</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ BALB/c mice were treated with polyclonal anti-ASGM1 and splenic NK cell function assessed as described in Materials and Methods 1.5 and 7 days later (data not shown), with similar results.
Table III. Chronic in vivo administration of anti-NK 1.1. mAb results in functional and phenotypic depletion of NK1.1-expressing spleen cells in C57BL/6

<table>
<thead>
<tr>
<th>In Vivo Treatment</th>
<th>Specific Cytotoxicity (% at 100:1, E:T)</th>
<th>Lytic Units/mL (per 10^7 cells)</th>
<th>Functional Analysisa</th>
<th>Phenotypic Analysisb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19</td>
<td>71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK136</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (poly I:C)</td>
<td>47</td>
<td>110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PK136 (poly I:C)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a NK cell function assessed using whole spleen cell preparations 24 h following i.p. injection of 300 μg PK136 mAb. Similar results were obtained following sacrifice of mice 7 days posttreatment (data not shown).

b FACS analysis using biotinylated anti-NK1.1 or isotype-matched biotinylated irrelevant mAb followed by Streptavidin-PE.

In vivo depletion of NK1.1-expressing cells does not inhibit induction of OVA-specific cytokine or Ab responses

As ASGM1 is expressed on rare peripheral T cells and monocytes as well as NK cells (41), an in vivo NK cell depletion model developed by Koo and colleagues (42) was employed as a third approach. We took advantage of the fact that B6 mice constitutively exhibit NK cell activity that is higher than that of BALB/c and that can be selectively abolished by chronic administration of anti-NK 1.1 mAb. Anti-NK1.1-treated C57BI/6 mice exhibited no detectable NK cell lytic function, even in groups administered poly(I:C) 18 h before sacrifice, indicating the effectiveness of this approach (Table III). Phenotypic analysis further confirmed its efficacy in depleting NK1.1-expressing cells among whole spleen and CD4^+/-enriched populations to levels below the limits of detection (Fig. 3 and Table III). Finally, while anti-NK1.1 has been shown to be capable of activating IFN-γ synthesis in vitro in the absence of other stimuli (43), spleen cells from mice treated with PK136 and their controls consistently demonstrated IFN-γ, IL-4, and IL-10 responses that were below the levels of detection (0.1–0.3 U/ml) when cultured without OVA restimulation (data not shown). Collectively, the data demonstrate that chronic, high dose, in vivo administration of PK136 reduces classical NK and NK1.1 T cell activities to below detectable levels.

Examination of OVA-specific IgE production (with respect to intensity or kinetics) in NK-depleted mice revealed no differences relative to that in untreated or normal mouse IgG-treated controls (Fig. 4). Development and maintenance of primary and secondary IgG1 and IgG2a responses were similarly unaffected by anti-NK1.1 treatment. Direct analysis of cytokine synthesis in bulk culture also failed to support the contention that depletion of NK1.1-expressing populations impacts on primary cytokine responses in this system in terms of either the strength or the relative Th1/Th2 balance of the OVA-specific response (Fig. 5). Reasoning that the kinetics, rather than the balance or the peak intensity, of the IFN-γ or IL-4/IL-10 responses might be influenced by NK cell depletion, OVA-stimulated bulk cultures were
established at 1, 2, 3.5, 5, and 8 days postimmunization (Fig. 6). IL-4, IL-10, and IFN-γ responses in OVA-immunized mice are very low (0.5–4 U/ml) or undetectable on day 1 (not shown) and day 2 postimmunization. Ag-driven cytokine responses increase by day 3.5, reaching their maximum on day 5. Neither the speed with which responses developed in vivo nor the intensity at any of the time points examined differed among the groups. In the absence of OVA restimulation in vitro, each of the cytokines examined was at undetectable levels (data not shown). Taken as a whole, the data argue that NK cells and NK1.1 T cells do not play an essential role in shaping the establishment or maintenance of the Th1/Th2 balance or the expression of in vivo effector responses, such as serum Ab.

Discussion

Recognition that stimulation of NK cells and NK1.1 T cells can elicit rapid, intense IFN-γ and IL-4/IFN-γ production, respectively, even when these cells are in a resting, nonpreactivated state, suggests a potential role for these cells as important early sources of cytokine driving commitment to Th1- and Th2-dominated responses. Using three independent experimental methods, we demonstrate that the absence of detectable NK or NK1.1 T cells does not discernibly influence the development of murine cytokine (IFN-γ vs IL-4 and IL-10) or serum Ab (IgG2a vs IgE) responses elicited by soluble protein Ag.

In vivo studies indicating that NK cells secrete IFN-γ early during the course of bacterial infection without a requirement for T cell help (18), in vitro studies demonstrating a capability of activated NK cells to increase IgG2a synthesis (44, 45), and findings that superantigens can act directly on NK cells to activate IFN-γ synthesis (23) are consistent with hypotheses linking innate and acquired immunity in the development of T cell-dependent responses (11–14, 31). Compelling evidence of a role for NK cells in shaping the initial induction of Th1-like responses in vivo has been obtained for several bacterial, protozoal, and viral pathogens, suggesting a potent mechanism by which the innate immune response could limit the development of potentially hazardous Th2-like cytokine production. Conversely, the capacity of NK1.11 T cells to promptly produce IL-4 following polyclonal activation (27–32) is frequently suggested as the source of the initial burst of IL-4 that is needed to steer commitment of conventional CD4 T cells to Th2-dominated patterns. Studies indicating that transgenic mice with marked overexpression of NK1.1 T cells exhibited 10- to 100-fold increases in IL-4 production (following mitogen-driven activation) in vitro or in vivo and that baseline levels of IgE and IgG1 were selectively elevated in vivo were consistent with this hypothesis (29).

The novelty of our approach lies in its focus on the role of NK cells and NK1.1 T cells in the development of soluble Ag-driven responses; the class of Ag that is of primary relevance to immediate hypersensitivity. We initially hypothesized that depletion of classical NK cells before immunization would lead to less Th1-like and more Th2-like cytokine and Ab responses. Specifically, we reasoned that if NK cells play an essential role in shaping the developing OVA-specific response, their depletion immediately
before Ag exposure should markedly influence both cytokine synthesis in short term culture and the balance of OVA-specific IgE vs IgG2a synthesis in vivo. Unseparated spleen cell populations were used for culture to avoid excluding, a priori, contributions to cytokine synthesis by non-CD4 cell populations.

The first approach to test this hypothesis, using normal and bg/bg C57Bl/6 mice, demonstrated strong, qualitatively identical (with respect to Th1-like vs Th2-like activation) cytokine and Ab responses following OVA immunization. Administration of anti-ASGM1 or normal rabbit IgG to BALB/c mice yielded similar conclusions. The third, most specific, approach used anti-NK1.1 treatment to evaluate the impact of NK cells and NK1.1-expressing cells on initial development of soluble Ag-driven responses. Again, OVA-specific IgE, IgG1, and IgG2a production (intensity or kinetics) revealed no differences between treated and control mice. Each of the experimental models used has recognized limitations. The concerns associated with bg/bg mice are well recognized. Anti-ASGM1 treatment may deplete low frequency subpopulations of activated T cells that also express these markers. Treatment with lower concentrations of anti-NK1.1 (i.e., one dose of 50 μg, i.p.) yields virtually complete elimination of classical NK cells but only partial removal of NK1.1 T cells (35). Notwithstanding these reservations, we note that each of the models used above has been used in support of a role for NK cells in directing the acquired immune response.

Alternative approaches to NK cell inactivation, such as chronic administration of anti-IL-2R β-chain mAb (46), were not evaluated in this study due to our concern that they would have additional, less specific, effects on the immune response (37).

Others have shown that NK cell depletion via anti-ASGM1 or anti-NK1.1 treatment before immunization or infection led to markedly decreased IFN-γ production and/or increased IL-4 synthesis following short term Ag-driven restimulation in vitro (13, 19–21). These qualitative changes in the dominant pattern of cytokine responsiveness were frequently accompanied by substantial changes in effector functions, such as serum Ab responses or host survival in response to pathogen challenge, providing support for the hypothesis that NK cells act as an essential link between innate and adaptive immunity to these pathogens. We speculate that the difference between these studies and our own may be in the nature of the inducing Ags and, consequently, the APC populations used to initiate the CD4 responses measured.
The role of NK cells in shaping responsiveness to soluble protein Ags was initially considered by Bogen et al. (12). Immuno-histochemical evidence was taken to suggest the presence of IFN-γ-producing NK-like cells at 3 and 7 days postimmunization of mice injected in the footpads with Ag in CFA or, to a lesser extent, aluminum hydroxide. Anti-ASGM1-mediated NK cell depletion reduced KLH-specific IgG2a responses by two- to threefold, unlike the data reported here or that reported by Wilder et al. (45) for anti-NK1.1-treated B6 mice. Bogen speculated that this NK-like response regulates the phenotype of the subsequent phosphorylcholine specific T cell response by promoting the development of Th1 cells. Subsequent efforts to evaluate the impact of anti-NK1.1 treatment on cytokine and Ab responsiveness in B6 mice were unsuccessful in obtaining NK cell depletion (25).

Although most attention has centered on the hypothesis that NK cells produce IFN-γ early, thereby promoting a default Th0/Th1-like response in preference to a potentially hazardous Th2 response, several groups have (27–32) recently published evidence demonstrating that high levels of IL-4 (and IFN-γ) are readily derived from low frequency NK1.1+CD4−/−CD3− peripheral cells. In at least one in vivo model, NKT cells were shown to be essential for generation of a Th2 response (27). These data have been taken by some groups to conclude that class I-selected CD4−CD8− and CD4+CD8+ aNKT T cells are important sources of IL-4 in the development of specific Th2 immune responses in mice (28, 31) and, perhaps, humans (47). The mechanism by which these class I-restricted T cell populations with highly restricted TCR repertoires are activated in vivo (i.e., other than by experimental treatment on cytokine and Ab responsiveness in B6 mice were unsuccessful in obtaining NK cell depletion (25).

In summary, while numerous investigators have used in vivo depletion models of NK cell activity to argue for a critical role for NK cells as a link between innate and acquired resistance, the data above and data that depletion of classical NK cells or of T cell populations expressing NK1.1-associated markers neither inhibits nor selectively promotes Th1- or Th2-like activation in the OVA-specific response. While there is strong evidence of an important role for this arm of the innate immune response in shaping host resistance to many pathogens or T-independent Ags (54, 55), the role of NK cells in influencing effector responses to exogenous protein Ags appears minor.

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References