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NK Cells Promote Type 1 T Cell Immunity through Modulating the Function of Dendritic Cells during Intracellular Bacterial Infection

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Dendritic cells (DC) play a key role in establishing protective adaptive immunity in intracellular bacterial infections, but the cells influencing DC function in vivo remain unclear. In this study, we investigated the role of NK cells in modulating the function of DC using a murine *Chlamydia* infection model. We found that the NK cell-depleted mice showed exacerbated disease after respiratory tract *Chlamydia muridarum* infection, which was correlated with altered T cell cytokine profile. Furthermore, DC from *C. muridarum*-infected NK-depleted mice (NK−DC) exhibited a less mature phenotype compared with that of DC from the infected mice without NK depletion (NK+DC). NK−DC produced significantly lower levels of both IL-12 and IL-10 than those of NK+DC. Moreover, NK−DC showed reduced ability to direct primary and established Ag-specific Th1 CD4+ T cell responses in DC−T coculture systems. More importantly, adoptive transfer of NK−DC, in contrast to NK+DC, failed to induce type 1 protective immunity in recipients after challenge infection. Finally, NK cells showed strong direct enhancing effect on IL-12 production by DC in an NK−DC coculture system, which was partially reduced by blocking NKG2D receptors signaling and virtually abolished by neutralizing IFN-γ activity. The data demonstrate a critical role of NK cells in modulating DC function in an intracellular bacterial infection. *The Journal of Immunology*, 2011, 187: 401–411.

*Chlamydia* are obligate intracellular bacterial pathogens that cause a wide spectrum of human diseases worldwide, ranging from ocular inflammation to pneumonia to sexually transmitted diseases (1). Because of insufficient understanding of the mechanisms underlying the development of pathogenesis or host protective response to chlamydial infection, an effective human vaccine is not available at the present time. It has been recognized that the failure of *Chlamydia*-specific T cell immunity is one of the most important factors by which *Chlamydia* establishes its persistence and causes tissue damage (reviewed in Refs. 2, 3). Th1-dominated immune responses and IFN-γ have been well documented as the major protective factors in the resolution of chlamydial infection (reviewed in Refs. 2, 3). As the most potent APC, dendritic cells (DC) are regarded as an important linkage between innate and adaptive immune responses. After sensing pathogens, immature DC undergo a maturation process, characterized by upregulation of costimulatory molecules and proinflammatory cytokines, and migrate to draining lymph nodes, where DC can initiate and direct T cell responses (4–6). The development of adaptive immune responses is largely dependent on DC phenotype and function, which may be regulated by the innate immune cells and pathogen-associated molecular patterns.

NK cells are one of the key components of the innate immune system involved in the defense against invading microbial pathogens. The important role of NK cells in host defense to infection has been demonstrated in several infection models including viral and intracellular bacterial infections (7–9). Limited studies have reported inconsistent findings on the role of NK cells in host immune response to chlamydial infections especially with different model systems (10–15). In murine genital tract chlamydial infection, NK cells are found to be potent producers of IFN-γ at the early stages of infection; NK cells are recruited into genital epithelium at early time points and may recognize/lyse infected cells (10). It has been reported that *Chlamydia trachomatis* infection decreases the expression of MHC class I molecules thereby increasing the susceptibility of *Chlamydia*-infected cells to be lysed by NK cells (13). However, in the studies using lung infection models and genetically modified animals, it was found that the role of NK cells in host defense against chlamydial infection was negligible (12, 14, 15). Even in the studies showing a protective role of NK cells in host defense against chlamydial infection (10–12), the mechanism(s) remains largely unclear.

A line of evidence indicates that NK cells can regulate DC function and that NK−DC cross-regulation plays an important role in dictating the quality and the quantity of the adaptive immune responses (16–21). A recent study showed that NK cells can enhance the capacity of DC to present parasitic Ags via the NKG2D receptor pathway (21). The important role of DC in initiation and maintenance of T cell responses to chlamydial infection has been demonstrated by several previous studies using mouse models of *C. trachomatis* mouse pneumonitis strain, more recently called *Chlamydia muridarum* (22–31). Su et al. (22) reported that i.v. delivery of bone marrow-derived DC pulsed with killed *C. muridarum* induced strong Th1-type protective immunity to genital tract challenge. Similarly, adoptive transfer of a cultured DC line...
NK cell depletion in vivo

In most of the experiments, NK cells in host defense against chlamydial lung infection. In particular, we focused on investigating the influence of NK cells on the function of DC especially for inducing protective T cell immunity against chlamydial infection. We found that depletion of NK cells significantly increased chlamydial lung infection. Moreover, we found that the NK cell depletion significantly altered the phenotypic and functional maturation of DC in chlamydial infection. DC from NK-depleted mice (NK–DC), unlike those from intact mice (NK+DC), showed reduced ability to direct Th1-type Ag-specific CD4+ T cell responses in vitro (DC–T coculture systems and failed to induce protective Th1 immunity after being adoptively transferred. Furthermore, NK cells isolated from C. muridarum-infected mice showed strong enhancing effect on IL-12 production by DC, which was partially reduced by blocking NKG2D receptor signaling and virtually abolished by neutralizing IFN-γ activity in an NK–DC coculture system. Thus, the current study demonstrates a novel role of NK cells in the development of protective adaptive immune responses to chlamydial infection by modulating DC function through IFN-γ production and receptor–ligand interactions.

Materials and Methods

Mice

Most of the study was performed using female C57BL/6 mice (6–8 wk old) that were bred at the University of Manitoba breeding facility. For DC–T cell coculture studies, breeding pairs of DO11.10 mice (BALB/c background, source of CD4 T cell) were purchased from The Jackson Laboratory and bred at the animal care facility of the University of Manitoba (Winnipeg, MB, Canada). The control BALB/c mice (source of DC) were bred at the University of Manitoba breeding facility. All the studies were performed in accordance with the guidelines issued by the Canadian Council on Animal Care, and the experimental protocols were approved by the University of Manitoba Animal Care and Use Committee.

Organism

The culture and propagation of C. muridarum were performed as described (3). Briefly, C. muridarum was grown in HeLa 229 cells in Eagle’s MEM containing 10% FBS and 2 mM l-glutamine for 48 h. For inoculum preparation, chlamydial elementary bodies were purified, tittered, and stored frozen at −80˚C in sucrose–phosphate–glutamic acid (SPG) buffer. The tissue fragments were transferred into 15-ml tubes and centrifuged. The cell pellet was resuspended in 5 ml 35% (v/v) Percoll (Pharmacia, Uppsala, Sweden) and centrifuged at 2000 rpm for 15 min at room temperature. RBCs were lysed with ACK lysis buffer followed by two washes in RPMI 1640 with 5% FCS and resuspended in proper medium or buffer according to different applications.

Spleen cell culture and cytokine measurements

Mice were sacrificed at various days p.i., and spleens were aseptically removed. Single-cell suspensions were prepared from spleens and cultured at a concentration of 7.5×106 cell/ml alone or with UV-killed C. muridarum (105 IFU/ml) in complete culture medium (RPMI 1640 containing 10% heat-inactivated FBS, 25 µg/ml gentamicin, 2 mM l-glutamine, and 5×10−5 2-mercaptoethanol) for 72 h. After incubation, supernatants were collected and measured for IL-4 and IFN-γ production by ELISA using purified (capture) and biotinylated (detection) Abs from BD Pharmingen as described previously (3).

Isolation of DC and cytokine analysis

Spleens were aseptically removed from mice at specific time points after C. muridarum intranasal infection. The lungs were cleared of blood by perfusion using ice-cold PBS before being collected from the chest. The lungs were cut into small pieces and digested for 1 h at 37˚C using 2 mg/ml collagenase XI (Sigma-Aldrich) and 100 µg/ml DNase I (Sigma-Aldrich). After digestion, the tissue fragments were transferred into 15-ml tubes and centrifuged. The cell pellet was resuspended in 5 ml 35% (v/v) Percoll (Pharmacia, Uppsala, Sweden) and centrifuged at 2000 rpm for 15 min at room temperature. RBCs were lysed with ACK lysis buffer followed by two washes in RPMI 1640 with 5% FCS and resuspended in proper medium or buffer according to different applications.

Histological analysis

Lungs from the infected mice were aseptically removed at indicated time points postinfection (p.i.) and fixed in 10% buffered formalin and embedded in paraffin for histological assessment as described previously (3). The tissue sections were stained with H&E. The histological changes and cellular infiltration were determined by light microscopy.

Lung mononuclear cell preparation

Mice were aseptically sacrificed at specific time points after C. muridarum intranasal infection. The lungs were cleared of blood by perfusion using ice-cold PBS before being collected from the chest. The lungs were cut into small pieces and digested for 1 h at 37˚C using 2 mg/ml collagenase XI (Sigma-Aldrich) and 100 µg/ml DNase I (Sigma-Aldrich). After digestion, the tissue fragments were transferred into 15-ml tubes and centrifuged. The cell pellet was resuspended in 5 ml 35% (v/v) Percoll (Pharmacia, Uppsala, Sweden) and centrifuged at 2000 rpm for 15 min at room temperature. RBCs were lysed with ACK lysis buffer followed by two washes in RPMI 1640 with 5% FCS and resuspended in proper medium or buffer according to different applications.

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DC–T cell coculture

CD4+ T cells were isolated from naive DO11.10 OVA peptide-specific TCR-αβ transgenic mice. Briefly, splenocytes passed through two consecutive CD4 enrichment columns (MACS; Miltenyi Biotec, Auburn, CA) and MACS columns as previously described (3). The purity of the CD4 T cells (95%) was confirmed by flow cytometric analysis. Anti-CD4 mAb (eBioscience) was used in specified experiments. For depletion of CD4 T cells in vivo, anti-CD4 mAb (eBioscience) was injected intravenously (20 µg).

Mice infection and quantification of in vivo bacterial load

Mice were mildly sedated with isoflurane and inoculated intranasally with 1×107 inclusion-forming units (IFUs) of C. muridarum in 40 µl SPG buffer. Body weights of mice were monitored daily. At predetermined days after inoculation, the mice were euthanized and the lungs were aseptically isolated and homogenized using a cell grinder in 0.1 M PBS. The tissue homogenates were centrifuged at 1900 × g for 30 min at 4˚C, and the supernatants were stored at −80˚C until tested. For assessment of C. muridarum growth in vivo, 100 µl of serially diluted organ tissue supernatants was inoculated onto HeLa229 monolayers in 96-well flat-bottom microtiter plates. After 2 h of incubation, the cell layers were washed, and 200 µl MEM containing cycloheximide (1.5 g/ml), gentamicin (20 g/ml), and vancomycin (25 g/ml) was added to each well. The plates were incubated for 48 h at 37˚C in 5% CO2. After the incubation, the culture medium was removed, and cell monolayers were fixed with methanol and subsequently stained with Chlamydia-specific murine mAb and HRP-conjugated goat anti-mouse IgG secondary Abs and developed with substrate (4-chloro-1-naphthol, Sigma-Aldrich). The number of inclusions was counted under a microscope at ×200 magnification. Five fields through the midline of each well were counted. Chlamydial tilters in the organs were calculated based on dilution titer of the original inoculum. The days for analyzing bacterial loads and pathological changes included day 8 and/or day 12, which represented the peak and the relative late stage of infection, respectively.

Histological analysis

Lungs from the infected mice were aseptically removed at indicated time points postinfection (p.i.) and fixed in 10% buffered formalin and embedded in paraffin for histological assessment as described previously (3). The tissue sections were stained with H&E. The histological changes and cellular infiltration were determined by light microscopy.

Spleen cell culture and cytokine measurements

Mice were sacrificed at various days p.i., and spleens were aseptically removed. Single-cell suspensions were prepared from spleens and cultured at a concentration of 7.5×106 cell/ml alone or with UV-killed C. muridarum (105 IFU/ml) in complete culture medium (RPMI 1640 containing 10% heat-inactivated FBS, 25 µg/ml gentamicin, 2 mM l-glutamine, and 5×10−5 2-mercaptoethanol) for 72 h. After incubation, supernatants were collected and measured for IL-4 and IFN-γ production by ELISA using purified (capture) and biotinylated (detection) Abs from BD Pharmingen as described previously (3).

Isolation of DC and cytokine analysis

Spleens were aseptically removed from mice at specific time points after C. muridarum infection, and DC were isolated using anti-CD11c magnetic beads (Miltenyi Biotec, Auburn, CA) and MACS columns as previously described (3). The purity was >95% as determined by flow cytometric analysis. Freshly isolated DC were cultured at 1×106 cell/ml in 96-well plates in the presence of UV-killed C. muridarum (1×105 IFU/ml) for 48 h of incubation, supernatants were collected, and the levels of IL-12, IL-10, and IL-6 were determined by ELISA using purified (capture) and biotinylated (detection) Abs (eBioscience) as described (3).

DC–T cell coculture

CD4+ T cells were isolated from naive DO11.10 OVA peptide-specific TCR-αβ transgenic mice. Briefly, splenocytes passed through two consecutive CD4 enrichment columns (MACS; Miltenyi Biotec). The purity of the CD4 T cells (95%) was confirmed by flow cytometric analysis. Anti-CD4 mAb (eBioscience) was used in specified experiments. For depletion of CD4 T cells in vivo, anti-CD4 mAb (eBioscience) was injected intravenously (20 µg).

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Abs in complete medium [RPMI 1640 supplemented with 10% FBS, 25 g/ml gentamicin, and 5 × 10^-5 M 2-mercaptoethanol (Kodak, Rochester, NY)] for 72 h at 37°C with 5% CO₂, and supernatants were harvested for cytokine (IL-4, IL-5, IL-10, and IFN-γ) assay by ELISA.

To determine the ability of DC in activating organism-specific CD4⁺ T cell response, T cells were isolated from Chlamydia-immunized mice. For immunization, C57BL/6 mice were injected with 1 × 10⁵ IFU C. muridarum i.p. and 2 wk later boosted with the same dose of infection (32). One week after the challenge, CD4⁺ T cells were isolated from spleens of C. muridarum-immunized mice (C. muridarum-specific CD4⁺ T) or naive (CD4⁺ T) mice using anti-CD4 magnetic beads (Miltenyi Biotec) according to the manufacturer’s instructions. CD11c⁺ DC were isolated from naive or C. muridarum-infected mice with anti-asialo-GM1 or control IgG treatment at day 12 p.i. DC (0.5 × 10⁵ to 1 × 10⁵) were cocultured with CD4⁺ T cells (5 × 10⁶) in 200 µl complete culture medium with the stimulation of UV-killed C. muridarum (1 × 10⁵ IFU/ml) in 96-well plates. The concentration of IFN-γ in supernatant was measured by ELISA. The controls include culture of CD4⁺ T cells alone, DC alone, DC coculture with naive CD4⁺ T cells and DC/CD4⁺ T cell coculture without antigenic stimulation.

DC–NK cell coculture

To isolate NK cells, T cells in the total spleen cells suspension were first depleted by using FITC-conjugated CD3e and anti-FITC microbeads as well as MACS depletion columns (Miltenyi Biotec). Briefly, 10 µl CD3e–FITC Ab was incubated with 10⁶ spleen cells for 20 min on ice. After wash, 10 µl anti-FITC microbeads were incubated with 10⁶ spleen cells for 20 min on ice followed by running through the MACS depletion column to deplete the T cells. NK cells were then isolated by positive selection by incubating the T cell-depleted spleen cells with anti-CD5 DX5 beads. The purity of NK cells was >95% according to the flow cytometric analysis. Purified NK cells (2 × 10⁵) isolated from C. muridarum-infected mice (day 3 p.i.) were cocultured with DC from naive syngeneic mice (2 × 10⁵) in the presence of UV-killed C. muridarum (1 × 10⁵ IFU/200 µl) in 96-well plates with or without 50 µg/ml anti–IFN-γ or 3 µg/ml anti-NKG2D Abs (Armenian Hamster IgG purchased from eBioscience). The IL-12 production levels in the 48-h culture supernatants were measured by ELISA. In the indicated experiment, NK cells and DC were physically separated in the coculture by using Transwell plates (Corning) with an 0.4-µm-pore membrane, which allows the free movement of soluble molecules but prevents direct contact between these two cells. DC was seeded in the lower chamber, whereas NK cells were placed on the top and UV-killed C. muridarum were added in both chambers.

Flow cytometric analysis

Anti-CD3e–PE–Cy7, anti-CD3e–PE, anti-CD4–PE, anti-CD6–PE–Cy7, anti-NK1.1–PE, anti-NK1.1–FITC, anti-CD11c–FITC, anti-MHCI–PE, anti-CD80–PE, anti-CD86–PE, and anti-CD8α–PE, and corresponding isotype controls were purchased from BD Biosciences. To examine the expression of NK cells after C. muridarum infection, freshly isolated splenocytes and pulmonary cells were stained with anti-CD3e–PE and anti-NK1.1–FITC. To examine the efficacy of anti-asialo-GM1 Ab treatment, splenocytes were stained using PE–NK1.1, FITC–CD3ε, and PE–mCD8/PBS57 ligand tetramer (generously provided by the National Institute of Allergy and Infectious Diseases MHC Tetramer Core Facility, Atlanta, GA). NK T cells were identified as tetramer- and CD3⁺ cell population. To evaluate the DC surface marker expression, DC isolated from the spleens of mice were stained by using anti-CD11c–FITC, anti-MHCII–PE, anti-CD80–PE, anti-CD86–PE, and anti-CD8α–PE as previously described (33). Analyses were performed by using a FACScalibur flow cytometer and CellQuest program (BD Biosciences).

Intracellular cytokine staining

Splenocytes and lung mononuclear cells (MNCs) were aseptically isolated from mice at predetermined time points after C. muridarum infection, incubated at 1 × 10⁶ cells/ml in 1 ml tissue culture and stimulated with 50-µl phorbol 12-myristate 13-acetate (Sigma Chemical) and 1 µg/ml ionomycin (Sigma). After 3 h of incubation, 20 µg/ml brefeldin A (Sigma) was added, and samples were incubated for another 3 h. After washing with staining buffer (Dulbecco’s PBS, 2%, heat-inactivated FBS, 0.05% sodium azide), cells were incubated with anti-mouse CD16/32 (Fc block; eBioscience) for 20 min on ice to block the Fc nonspecific binding and subsequently stained for surface markers with anti-NK1.1–FITC, anti-CD4–PE, anti-CD4–FITC, anti-CD3ε–FITC, anti-CD3ε–PE (BD Biosciences), or isotype controls for 30 min on ice. The cells were fixed and permeabilized with Cytofix/Cytoperm buffer (BD Biosciences) for 20 min at 4°C and incubated with anti-IFN-γ–allophycocyanin, anti–IL-4–allophycocyanin, or corresponding isotype controls (eBioscience) for 30 min on ice. The raw sample data were collected using a FACScalibur flow cytometer (BD Biosciences), and the data were analyzed using CellQuest software (BD Biosciences).

Results

C. muridarum infection induced rapid NK cell expansion and IFN-γ production

We first examined the kinetics of NK cell activation after C. muridarum lung infection. A notable increase of NK cell population was detected in both spleen and lung tissues from the infected mice at 24 h after infection (Fig. 1A, 1C). The number of NK cells was seen to peak at day 3 p.i. in both spleen and lung tissues. The frequency of IFN-γ–producing NK cells also increased significantly in the spleen and lung as indicated by intracellular cytokine staining analysis (Fig. 1B, 1D). Analysis of NK cells in peripheral blood also showed increase of NK cells after C. muridarum infection (data not shown). These data indicate that NK cells are activated rapidly during C. muridarum lung infection and produce IFN-γ in the early phase of infection.

NK cell depletion leads to reduced ability to clear C. muridarum infection, more severe tissue pathology, and decreased Th1 cellular response

To test the contribution of NK response in host defense against chlamydial infection, we selectively depleted NK cells by anti–asialo-GM1 Ab treatment of mice. To exclude the possibility that this treatment might affect in vivo NK T cell thus influencing the course of infection (32), we examined NK T cell population in the anti–asialo-GM1 Ab-treated mice. NK T cells were stained using CD1d tetramers as well as NK.1.1 Abs and analyzed by flow cytometry. As shown in the Fig. 2A and 2B, this treatment effectively depleted NK cells without affecting NK T cells. The treatment using control Ab (sham) had no effect on either NK or NK T cell population.

To confirm the functional involvement of NK cells in the host defense against C. muridarum infection, NK cell-depleted mice and control Ab-treated C57BL/6 mice (sham mice) were infected with C. muridarum, and the outcome of infection was evaluated. We found that NK cell depletion dramatically exacerbated the course of infection. After C. muridarum infection, NK cell-depleted mice showed more severe body weight loss and failed to recover compared with the sham mice, which began to regain the lost body weight from day 8 p.i. (Fig. 2A, 3A). In correlation with this observation, NK cell-depleted mice showed significantly higher bacterial load in their lungs than that of sham-treated mice at both earlier (day 7) and later (day 12) stages of infection (Fig. 2B). In addition to use of anti–asialo-GM1, we also used another Ab (anti-NK1.1) to deplete NK cells, which also showed more serious disease in the NK cell-depleted mice (Fig. 2C).

The lung histological analysis showed that NK cell-depleted mice experienced much more severe lung pathological changes
decreased IFN-γ production; the proportion of IFN-γ–producing cells was reduced to half compared with that of sham-treated mice on day 12 p.i. To confirm the relevance of CD4 T cell responses in protection in this NK cell depletion model, we further compared the effect of depletion of CD4 T cells alone and the depletion of both CD4 and NK cells. As shown in Fig. 4D, the double depletion of NK cells and CD4 T cells had a similar level of detrimental effect (increase of bacterial loads) to protection as depletion of CD4 T cells alone, confirming the importance of CD4 T cell responses on host defense against *C. muridarum* infection. In general, our data suggest that the alteration in host susceptibility to chlamydial infection observed in NK cell-depleted mice was at least partially related to altered T cell cytokine production due to depletion of NK cells. Altogether, these results suggest that NK cells play an important role in the host protective responses against *C. muridarum* lung infection.

**Depletion of NK cells affects DC maturation and function to induce Th1 CD4+ T cell response**

Having established that NK cells play an important role in influencing the development of protective CD4+ T cell response to *C. muridarum* infection, we further tested whether this is due to NK cell-mediated modulation of DC function. First, we examined the function of NK cells in the maturation of DC after *C. muridarum* infection. DC from NK cell-depleted and control groups of mice were analyzed for morphological features. Microscopic examination showed different morphologic features among DC isolated from naive (naive DC) mice and NK cell-depleted infected mice (NK DC) or mice treated with control IgG Ab and the same infection (NK'DC). NK DC exhibited more mature morphology (large rounded cells with longer dendrites) compared with that of NK DC, which displayed small rounded cells with less and short dendrites (not shown). The morphology of NK DC was similar to that of naive DC. We next analyzed whether depletion of NK cells affects the surface expression of maturation/costimulatory molecules on DC after *C. muridarum* infection. We previously have shown that DC express higher levels of CD80, CD86, and CD86 after *C. muridarum* infection (3). Consistently, NK+DC showed higher levels of expression of these surface molecules compared with those of naive DC (Fig. 5A, Table I). However, the expression of maturation and costimulatory molecules on NK DC p.i. was similar to that in naive DC (Fig. 5A, Table I). These results suggest that NK cells play a role in inducing the maturation of DC after *C. muridarum* lung infection.

Because cytokine production by DC is important for the function of DC in directing protective immune response to *C. muridarum* infection, we next analyzed whether NK cells influence DC cytokine production after infection. We examined the effect of NK cells on cytokine secretion by DC by comparing NK+DC, NK DC, and naive DC in ex vivo culture. As shown in Fig. 5B, NK+DC produced significantly higher levels of both IL-12 and IL-10 but less IL-6 compared with those of naive DC. In contrast, NK DC showed reduced IL-12 and IL-10 but not IL-6 production compared with that of naive DC (Fig. 5B).

To examine more directly whether NK cells play a role in regulating the functional ability of DC in priming naive T cell polarization, CD4+ T cells from DO11.10 transgenic mice, which expresses TCR specifically recognizing OVA, were cocultured with NK+DC, NK DC, or naive DC in the presence of OVA. The concentrations of cytokines in culture supernatants were measured by ELISA. As shown in Fig. 6A, NK DC compared with NK+DC had dramatically reduced ability to polarize OVA-specific naive CD4+ T cell to IFN-γ–producing cells. The data indicate that NK

**FIGURE 4.** IFN-γ production (Fig. 4D) after *C. muridarum* infection. C57BL/6 mice were intranasally infected with *C. muridarum* (1 × 10^7 IFU) and killed at various days p.i. The percentage of NK cells in living lymphocytes according to forward and side scatter (x-axis is NK1.1; y-axis is side scatter). Splenic (B) and lung (D) IFN-γ–producing NK cells in naive mice (left panel) and *C. muridarum*-infected mice (day 3 p.i.; right panel) were analyzed by intracellular cytokine staining. Fresh single spleen cell suspensions and pulmonary MNCs were stimulated for 6 h with PMA and ionomycin, with brefeldin A being added during the last 3 h, followed by FITC–anti-NK1.1 and PE–anti-CD3ε surface staining and allophycocyanin–anti–IFN-γ intracellular staining or corresponding isotype controls. The analysis was performed on NK cells gated as CD3+ NK1.1+ cells. Data are representative of one of three independent experiments.

A. IFN-γ (cells/10^6) and killing at various days p.i. The percentage of NK cells in CD3+ cells stained with FITC-labeled anti-NK1.1 and PE-labeled anti-CD3. Lung MNCs were prepared as described in Materials and Methods and stained with FITC-labeled anti-NK1.1 and PE-labeled anti-CD3ε mAb. Cells were gated on CD3+ living lymphocytes according to forward and side scatter (x-axis is NK1.1; y-axis is side scatter). Splenic (B) and lung (D) IFN-γ–producing NK cells in naive mice (left panel) and *C. muridarum*-infected mice (day 3 p.i.; right panel) were analyzed by intracellular cytokine staining. Fresh single spleen cell suspensions and pulmonary MNCs were stimulated for 6 h with PMA and ionomycin, with brefeldin A being added during the last 3 h, followed by FITC–anti-NK1.1 and PE–anti-CD3ε surface staining and allophycocyanin–anti–IFN-γ intracellular staining or corresponding isotype controls. The analysis was performed on NK cells gated as CD3+ NK1.1+ cells. Data are representative of one of three independent experiments.

B. IFN-γ (cells/10^6) and killing at various days p.i. The percentage of NK cells in CD3+ cells stained with FITC-labeled anti-NK1.1 and PE-labeled anti-CD3. Lung MNCs were prepared as described in Materials and Methods and stained with FITC-labeled anti-NK1.1 and PE-labeled anti-CD3ε mAb. Cells were gated on CD3+ living lymphocytes according to forward and side scatter (x-axis is NK1.1; y-axis is side scatter). Splenic (B) and lung (D) IFN-γ–producing NK cells in naive mice (left panel) and *C. muridarum*-infected mice (day 3 p.i.; right panel) were analyzed by intracellular cytokine staining. Fresh single spleen cell suspensions and pulmonary MNCs were stimulated for 6 h with PMA and ionomycin, with brefeldin A being added during the last 3 h, followed by FITC–anti-NK1.1 and PE–anti-CD3ε surface staining and allophycocyanin–anti–IFN-γ intracellular staining or corresponding isotype controls. The analysis was performed on NK cells gated as CD3+ NK1.1+ cells. Data are representative of one of three independent experiments.

C. IFN-γ (cells/10^6) and killing at various days p.i. The percentage of NK cells in CD3+ cells stained with FITC-labeled anti-NK1.1 and PE-labeled anti-CD3. Lung MNCs were prepared as described in Materials and Methods and stained with FITC-labeled anti-NK1.1 and PE-labeled anti-CD3ε mAb. Cells were gated on CD3+ living lymphocytes according to forward and side scatter (x-axis is NK1.1; y-axis is side scatter). Splenic (B) and lung (D) IFN-γ–producing NK cells in naive mice (left panel) and *C. muridarum*-infected mice (day 3 p.i.; right panel) were analyzed by intracellular cytokine staining. Fresh single spleen cell suspensions and pulmonary MNCs were stimulated for 6 h with PMA and ionomycin, with brefeldin A being added during the last 3 h, followed by FITC–anti-NK1.1 and PE–anti-CD3ε surface staining and allophycocyanin–anti–IFN-γ intracellular staining or corresponding isotype controls. The analysis was performed on NK cells gated as CD3+ NK1.1+ cells. Data are representative of one of three independent experiments.

D. IFN-γ (cells/10^6) and killing at various days p.i. The percentage of NK cells in CD3+ cells stained with FITC-labeled anti-NK1.1 and PE-labeled anti-CD3. Lung MNCs were prepared as described in Materials and Methods and stained with FITC-labeled anti-NK1.1 and PE-labeled anti-CD3ε mAb. Cells were gated on CD3+ living lymphocytes according to forward and side scatter (x-axis is NK1.1; y-axis is side scatter). Splenic (B) and lung (D) IFN-γ–producing NK cells in naive mice (left panel) and *C. muridarum*-infected mice (day 3 p.i.; right panel) were analyzed by intracellular cytokine staining. Fresh single spleen cell suspensions and pulmonary MNCs were stimulated for 6 h with PMA and ionomycin, with brefeldin A being added during the last 3 h, followed by FITC–anti-NK1.1 and PE–anti-CD3ε surface staining and allophycocyanin–anti–IFN-γ intracellular staining or corresponding isotype controls. The analysis was performed on NK cells gated as CD3+ NK1.1+ cells. Data are representative of one of three independent experiments.
cells are important for the regulation of DC ability to prime naive CD4+ T cells for Th1 response.

Further, we tested the effect of NK cells on DC ability to activate CD4+ T cell responses for *C. muridarum*-specific responses. *C. muridarum*-specific CD4+ T cells isolated from *C. muridarum*-immunized mice were cocultured with NK+DC, NK-DC, or naive DC in the presence or absence of killed *C. muridarum* stimulation. CD4+ T cells from naive C57BL/6 mice (naive T) were used as controls. As shown in Fig. 6B, CD4+ T cells showed much higher levels of Ag-driven IFN-γ production when cocultured with NK+ DC than with NK- DC or naive DC. Such effect on IFN-γ production was not observed with CD4+ T cells cultured with NK- DC. Collectively, these results demonstrate that NK cells modulate the phenotype and function of DC after infection with *C. muridarum*.

**FIGURE 2.** Depletion of NK cells by anti–asialo-GM1 Ab treatment had no effect on NKT cell population. *C. muridarum*-infected mice were treated with control IgG (sham) or anti–asialo-GM1 Ab (NK-). A, Flow cytometric analysis verifying NK cells depletion. Freshly isolated spleen cells at day 4 p.i. were stained with PerCP-labeled anti-CD3e and PE-labeled anti-NK1.1. Cells were gated on living lymphocytes according to forward and side scatter. *Left top squares* show NK cells (CD3- NK1.1+), and the *right lower square* shows NKT cells (CD3+ NK1.1+). B, CD1-d tetramer staining for NKT cell population. Spleen cells isolated at day 4 p.i. were stained with PE–Cy7–labeled anti-CD3e Abs and PE-labeled mCD1d/PBS57 ligand tetramer. The NKT cells were identified as CD3+ tetramer (+) cells by flow cytometry.

**FIGURE 3.** NK cells depletion leads to reduced ability to clear *C. muridarum* lung infection. C57BL/6 mice (four mice per group) were treated with control IgG (sham) or anti–asialo-GM1 (NK-) Ab during *C. muridarum* lung infection as described in Materials and Methods. Mice were sacrificed at day 12 p.i. A, More body weight loss after chlamydial infection in NK cells-depleted mice (NK-). Mice were monitored daily for body weight changes. Each point represents the mean ± SD of four mice. One representative experiment of four independent experiments with similar results is shown. B, More chlamydial growth in vivo after *C. muridarum* infection in mice with NK cell depletion (NK-). Lungs were homogenized and tested for bacterial loads as previously described (3). C, Relatively higher neutrophil but low lymphocytes were found in the lung. Cellular composition of the infiltrating cells in the lung histological slides was examined under microscope (original magnification ×400). The data of day 8 (D8) and day 12 (D12) are shown. D, Depletion of NK cells using anti-NK1.1 Ab also increased chlamydial loads in vivo. The chlamydial loads were shown as IFU. The mean of the log10 transformed IFU per lung is depicted. The results are shown as mean ± SD. One representative experiment of four independent experiments with similar results is shown. *p < 0.05, **p < 0.01, ***p < 0.001.
Adoptive transfer of NK−DC, unlike NK+DC, failed to protect against challenge infection

To investigate whether NK cell depletion during infection affects the ability of DC to induce protective immune responses against C. muridarum infection in vivo, DC from naive or infected mice with or without NK depletion were adoptively transferred to naive C57BL/6 mice recipients. All recipient mice were infected intranasally with C. muridarum 48 h after DC transfer. The body weight loss, in vivo bacterial growth, lung pathology, and cytokine profiles were analyzed. Mice that received NK+DC showed the least body weight loss (Fig. 7A) and lung bacterial load (Fig. 7B) among the three groups of mice. In contrast, mice transferred with NK−DC showed dramatic body loss and, in contrast to the recipients of naive DC or NK+DC, the mice had no sign of recovery at day 12 post-challenge infection when the mice were killed (Fig. 7A). In addition, significantly higher bacterial load in the lungs was observed in recipients of NK−DC than that in NK+DC recipients and also naive DC recipients (Fig. 7B). In correlation, histopathological analysis showed most severe inflammatory and pathological changes in the lungs of NK−DC recipients among the three groups (Fig. 7C). Further, the cytokine production was altered in these mice compared with mice that received NK+DC. Specifically, intracellular cytokine staining analysis showed a smaller number of IFN-γ–producing CD4+ T cells in the recipients of NK−DC than that in mice receiving NK+DC (Fig. 7D). Consistently, bulk culture of splenocytes isolated from mice that received NK−DC showed significantly decreased production of Chlamydia-driven IFN-γ production than that of those from NK+DC recipients (data not shown). Thus, NK cell deficiency reduced the ability of DC to induce protective CD4+ T cell responses after C. muridarum infection. The mice without cell transfer (PBS treatment alone) showed similar changes in body weight loss, bacterial load, and cytokine responses as recipients of naive DC (data not shown). In aggregate, these findings confirmed the protective effect of NK cells by modulating DC function to elicit efficient adaptive immunity in host defense to C. muridarum lung infection in vivo.

NK cells from Chlamydia-infected mice enhanced IL-12p70 production by DC through NKG2D receptor signaling and IFN-γ production

To examine directly the modulating effect of NK cells on DC and possible underlying mechanism, we purified NK cells from C. muridarum-infected mice (day 3 p.i.) and cocultured them with

FIGURE 4. NK cells depletion leads to altered pattern of T cell cytokine production after chlamydial infection. C57BL/6 mice (four mice per group) were treated with control IgG (sham) or anti-asialo-GM1 (NK−) Ab during C. muridarum mouse pneumonitis (MoPn) lung infection as described in Materials and Methods. Mice were sacrificed at day 12 p.i. Mice without infection were used as control. A, Freshly isolated splenocytes from individual mice without pooling were cultured for 72 h with stimulation of UV-killed C. muridarum. IFN-γ and IL-4 production in cell culture supernatants was determined by ELISA. The experiment is representative of three separate experiments. Each point represents the mean ± SD. B, IFN-γ and IL-4 production by splenic CD4+ T cells by intracellular cytokine staining. Freshly isolated splenocytes were stained by PE-conjugated anti-CD4, FITC-conjugated anti-CD3ε, and allophycocyanin-conjugated anti-IL-4 and anti-IFN-γ. Cells were gated on CD3+ living lymphocytes. C, Summary of the IFN-γ– and IL-4–expressing CD4 T cells in each group is shown as mean ± SD. D, Depletion of CD4 T cells alone or in combined with depletion of NK cells showed similar effect on chlamydial growth in the lung. The chlamydial growth in the lungs of each group is indicated as mean ± SD. One of three independent experiments with similar results is shown. *p < 0.05, **p < 0.01.
DC from naive mice in the presence of killed *C. muridarum*. The results showed that the addition of NK cells isolated from infected mice dramatically increased IL-12p70 production by cultured DC (Fig. 8A). The addition of NK cells from naive mice had no significant effect (data not shown). The blockade of physical contact between NK cells and DC in the coculture system using the Transwell significantly, but only partially, reduced the enhancing effect of NK cells on IL-12p70 production by DC (Fig. 8A). The data demonstrate that cell–cell contact is helpful for the enhancing effect of NK cells on DC but is not indispensable. Soluble proteins/factors released by NK cells may be involved in the DC–NK interaction. The blockade of NKG2D receptors using rat anti-mouse NKG2D mAb also led to partial reduction of the enhancing effect, similar to the blockade of cell–cell contact by Transwell culture (Fig. 8A). The NKG2D receptor is one of the important activating receptors on NK cells, and this mAb can block binding of NKG2D to its ligands and inhibits NKG2D-dependent NK cell-mediated function. In contrast, neutralization of IFN-γ activity in the coculture virtually completely abolished the enhancing effect of NK cells on IL-12p70 production by DC, suggesting a predominant role of IFN-γ production for the modulating effect (Fig. 8B). The results provide direct evidence that NK cells activated by chlamydial infection can modulate the Th1-promoting function of DC (IL-12p70 production) and that IFN-γ production and NKG2D signaling are critically important for the modulating effect of NK cells on DC.

**Discussion**

A large body of evidence show that activation of NK cells is integral for the effective control of infections and tumors insults (34, 35). In terms of the contribution of the NK responses in host defense against chlamydial infection, inconsistent results have been reported (10–15). In the current study, we performed a comprehensive investigation to examine the responses and function

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**Table I. Summary of flow cytometric analysis of surface markers expressed on DC (mean ± SEM)**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Naive DC (n = 16)</th>
<th>NK+DC (n = 16)</th>
<th>NK−DC (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>19.1 ± 5.3</td>
<td>40.8 ± 6.1</td>
<td>29.1 ± 7.1</td>
</tr>
<tr>
<td>MFI</td>
<td>19.9 ± 4.1</td>
<td>57.6 ± 5.9</td>
<td>21.6 ± 8.5</td>
</tr>
<tr>
<td>CD86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>17.1 ± 4.3</td>
<td>37.3 ± 6.2</td>
<td>21.7 ± 4.7</td>
</tr>
<tr>
<td>MFI</td>
<td>13.7 ± 4.8</td>
<td>38.8 ± 7.1</td>
<td>19.2 ± 7.4</td>
</tr>
<tr>
<td>MHC class II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>70.5 ± 11.9</td>
<td>81.3 ± 16.9</td>
<td>76.4 ± 9.9</td>
</tr>
<tr>
<td>MFI</td>
<td>651 ± 91.8</td>
<td>825.2 ± 118.2</td>
<td>734.6 ± 167.2</td>
</tr>
<tr>
<td>CD8α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>18.1 ± 6.3</td>
<td>32.7 ± 9.6</td>
<td>21.5 ± 3.5</td>
</tr>
<tr>
<td>MFI</td>
<td>108.2 ± 63.7</td>
<td>297.4 ± 70.4</td>
<td>127.5 ± 67.2</td>
</tr>
</tbody>
</table>

Statistical analysis to compare the expression of surface markers on naive DC, NK+DC, and NK−DC was performed using ANOVA test, followed by Newman-Keuls multiple comparison test. Results are pooled data from four experiments.

*p < 0.05, comparison between naive DC and NK+DC.

**p < 0.01, comparison between naive DC and NK+DC.

*p < 0.05, comparison between NK+DC and NK−DC.

*p < 0.01, comparison between NK+DC and NK−DC.

MFI, mean fluorescence intensity.
of NK cells during chlamydial lung infection. By performing a flow cytometric analysis, we found that NK cells expanded rapidly and produced high levels of IFN-\(\gamma\) after \(C.\) muridarum lung infection. More importantly, we demonstrated that NK cell deficiency led to increased susceptibility to \(C.\) muridarum lung infection characterized by increased organism growth in vivo, more severe pathology, and decreased protective cellular responses. More importantly, our results showed that T cell response,

**FIGURE 6.** Altered function of DC from NK cell-depleted mice to direct Ag-specific CD4\(^+\) T cell cytokine production. DC were isolated from spleens of naive mice (naive-DC) or \(C.\) muridarum mouse pneumonitis (MoPn)-infected mice with sham (NK\(^+\)DC) or NK cell depletion (NK\(^-\)DC) treatment at day 12 p.i. A, Naive CD4\(^+\) T cells were purified from spleens of OVA-specific TCR transgenic (DO11.10) mice. CD4\(^+\) T cells (5 \times 10^5/100 \mu l) were then cocultured with 5 \times 10^7/100 \mu l DC with the stimulation of OVA for 72 h in culture medium in 96-well plate. The concentrations of IL-4, IL-5, and IFN-\(\gamma\) in supernatants were examined by ELISA. B, IFN-\(\gamma\) production by \(C.\) muridarum-specific CD4\(^+\) T cells in DC–T coculture system. Organism-specific CD4\(^+\) T cells isolated from \(C.\) muridarum-immunized and challenged mice were cocultured with DC from naive or infected mice with or without NK cell depletion in the presence of UV-killed \(C.\) muridarum stimulation. CD4\(^+\) T cells from naive C57BL/6 mice were used as controls. The concentration of IFN-\(\gamma\) in supernatant was measured by ELISA. The results are shown as mean \(\pm\) SD. One of three similar experiments is shown. *\(p\) < 0.05, **\(p\) < 0.005.

**FIGURE 7.** Adoptive transfer of NK DC, unlike NK\(^+\)DC, fails to protect against \(C.\) muridarum challenge infection. Naive recipient mice (C57BL/6) were delivered DC isolated from spleens of naive mice (naive-DC), \(C.\) muridarum-infected, NK cell-depleted mice (NK\(^-\)DC), or \(C.\) muridarum-infected mice without NK cell depletion (NK\(^+\)DC), followed by intranasal challenge infection with the same chlamydial strain (1 \times 10^3 IFU) 48 h after DC transfer. All the mice were sacrificed at day 8 p.i. A, More body weight loss in mice receiving NK\(^-\)DC. B, The pulmonary bacterial load. Lungs of recipient mice were homogenized and tested for \(C.\) muridarum loads at day 8 p.i. The mean of the log_{10} transformed IFUs per lung is depicted. C, The pulmonary pathology. Lungs from recipient mice at day 8 and day 12 p.i. were sectioned and the pulmonary pathology examined by H&E staining and analyzed in low-power (\(\times 400\)) and high-power (\(\times 1000\)) magnification under light microscopy. D, Determination of IFN-\(\gamma\)-producing CD4\(^+\) T cell from spleen by intracellular staining. Cells were gated on living CD3\(^+\) cells. The IFN-\(\gamma\) CD4 T cells in the control mice without infection were \(<1\%\). *\(p\) < 0.05, **\(p\) < 0.01, ***\(p\) < 0.001.
was analyzed by ELISA. The results are shown as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

especially Th1 immunity, is greatly reduced in NK cell-depleted mice. Thus, NK cells influenced CD4+ T cells in the adaptive immune phase of C. muridarum infection, which has been demonstrated by our laboratory and by many others to be important for host defense against C. muridarum infection (36–38). The demonstration of the significant enhancing effect of NK cells on Th1 cells may provide an explanation for the observed unnecessary role of NK cells in host defense against chlamydial lung infection in some of the reported studies (12, 14, 15). Indeed, the observation of the failure of NK cells in protecting the host in chlamydial lung infection was largely obtained from the studies using T cell-deficient or T and B cells-deficient mutant or genetically modified animals (12, 14, 15). For example, one study (14) compared RAG-1−/−γγR−/− and RAG-1−/− mice, which both had deficiency of T and B cells, but one type (RAG-1−/−) had intact NK cells. The study found that these two types of gene knockout mice were similar in susceptibility to chlamydial infection, thus concluding that NK cells were not necessary for defense against the infection. However, because NK cells are more likely playing their role in host defense by influencing T cell responses (through modulating DC), rather than directly inhibiting C. muridarum, the presence of UV-killed C. muridarum only served as control. The supernatant was collected after 48-h culture, and IL-12p70 production was measured by ELISA.

**Figure 8.** NK cells from Chlamydia-infected mice enhanced IL-12p70 production by DC through cell–cell contact, NKG2D receptor signaling, and IFN-γ production. A. Effect of NK cells on IL-12p70 production by DC. Freshly isolated DC from naive C57BL/6 mice were cocultured with NK cells isolated from C. muridarum mouse pneumonitis (MoPn)-infected (day 3 p.i.) C57BL/6 mice (iNK) in the presence of UV-killed C. muridarum with or without blocking DC and NK cell contact (Transwell plate with 0.4-μm-pore membrane; Corning). DC cultured in medium alone or with UV-killed C. muridarum only served as control. The supernatant was collected after 48-h culture, and IL-12p70 production was measured by ELISA. B. Contribution of NKG2D signaling and IFN-γ production on the promoting effect of NK cells on IL-12p70 production by DC. Naïve DC were cocultured with NK cells isolated from infected mice at day 3 p.i. in the presence of UV-killed C. muridarum with or without the anti-NKG2D or anti–IFN-γ Ab for 48 h. The IL-12p70 concentration in the supernatant was analyzed by ELISA. The results are shown as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

The most significant finding in the study is the strong influence of NK cell response on DC function during chlamydial infection. The data confirmed and extended the previous findings on the cross-talk between NK cell and DC in models related to model Ags (18, 39), parasite infections (19, 20), and tumors (21). To elucidate the mechanism of how NK cells promote the development of efficient adaptive Th1 response, we have analyzed the DC phenotypic and functional characteristics after infection with C. muridarum. The type of DC response can profoundly affect the quality of subsequent development of adaptive immunity to infection. We directly examined the modulating effect of NK on DC using both in vivo and in vitro approaches. We found that DC from NK cell-depleted mice showed reduced expression of costimulatory molecules than that of the sham-treated mice (Fig. 5). Thus, DC maturation is favored by NK cell activity. Moreover, DC from NK cell-depleted mice produced significantly lower levels of both IL-12 and IL-10 but higher levels of IL-6 than those of DC from sham-treated mice after C. muridarum infection. Importantly, the changes in DC phenotype and cytokine production caused by NK cell depletion led to impairment of DC function in generating protective Th1 immunity. This is shown in both in vivo and in vitro experiments. In contrast to DC from sham-treated mice (NK+DC), which induced strong Ag-specific Th1 development (IFN-γ production) in both primary (Fig. 6A) and secondary (Fig. 6B) assays, the DC from NK cell-depleted mice (NK−DC) induced much less IFN-γ production by Ag-specific CD4+ T cells. When the data are examined for Th1/Th2 cytokine ratio, NK+DC promoted Th1 whereas NK−DC promoted Th2 response. More interestingly, the adaptive transfer of NK+DC, in contrast to NK−DC, promoted infection rather than generating protective immunity, demonstrated by more serious disease in NK−DC recipients than in naïve DC recipients and PBS-treated mice after challenge infection. The results demonstrate that NK cell may be critical in educating APC especially DC thereby augmenting the control of infection. This was also shown by the data showing that depletion of either CD4+ T cells alone or plus NK cells had similar levels of increase of infection, suggesting a limited function of NK cells per se in control chlamydial infection (Fig. 4D). The educating effect of NK cells on DC may 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. The lower IL-12 production and higher IL-6 production by NK+DC than NK−DC fit well with the impaired function of NK+DC in inducing Th1 response because IL-12 has been shown to be critical for Th1 response while IL-6 has been implicated in Th2 polarization of CD4+ T cells (40). However, the higher IL-10 production by NK+DC is rather surprising because IL-10 is often recognized as a cytokine without Th1 promoting activity. The data suggest that IL-12 may function in a predominant fashion when it coexists with IL-10. It is unclear whether these cytokines are produced by the same cell or by different subsets in the DC population. Notably, we recently reported that CD8α+ DC, which also produced high IL-12 and IL-10, induced stronger Th1 response than CD8α+ DC, which produced lower IL-12 and IL-10 (3). Further study needs to be performed on the relative contribution of IL-12 and IL-10, especially in the scenario of coexistence of higher levels of these two normally functionally different cytokines on T cell differentiation.

How does NK influence DC during chlamydial infection? Some recent studies have addressed the mechanisms by which NK cell might interact with DC (41–43). A recent study showed that NK cells can enhance the capacity of DC to present parasitic Ags via NKG2D receptor pathway (44). Using the NK−DC coculture approach, we demonstrated the direct influence of NK cells on DC...
function (Fig. 8). Notably, the modulating effect of NK cells on DC was involved in both cell–cell contact-dependent and -independent mechanisms. Specifically, the separation of NK cells and DC by membrane in the Transwell culture led to decreased IL-12–enhancing effect by NK cells but did not completely abolish the modulating effect (Fig. 8A). Consistently, the blockade of NKG2D receptor pathway significantly reduced the IL-12–enhancing effect of NK cells on DC but did not abolish the effect. In contrast, the blockade of IFN-γ activity completely abolished the modulating effect of NK cells on DC. The results suggest the critical importance of IFN-γ production by NK cells for their modulating effect on DC. This is consistent with the reports in other models that activated NK cells released cytokines that favored DC maturation, principally TNF-α and IFN-γ (41). Very likely, the influence of NK cells on DC involves many molecular interactions. For example, a study showed that the NK cell-activating receptor NKp30 played an important role in NK cell-mediated DC maturation or apoptosis in humans (45). Furthermore, recent studies (46) have reported that NK cells also mediate the elimination of immature DCs by cell contact-dependent interactions. These studies suggested that activated NK cells, by limiting the supply of immature DC, may then exert a control on subsequent innate and adaptive immune responses. It was also reported that NK cells could select most suitable DC for subsequent migration to lymph nodes and efficient T cell priming (42, 43). In addition, it has been shown that NK cells are important for the maintenance of CD8+ DC subset during mouse cytomegalovirus infection (47). We have shown in an earlier study that CD8+ DC are more DC1-like cells, thus inducing more protective Th1 responses than the CD8- DC in chlamydial infection (3). In the current study, we found that the depletion of NK cells in C. muridarum-infected mice resulted in decreased population of CD8+ DC. Thus, it is likely that NK cells promote the development of DC1-like cells during chlamydial infection.

Importantly, we showed the functional effect of NK cells on DC in both in vivo adoptive transfer experiments and in vitro coculture experiments. The adoptive transfer of DC from C. muridarum-infected mice to naive recipient mice resulted in a very dramatic reduction (nearly 10,000-fold) of bacterial loads compared with that of those without any DC transfer (data not shown) at day 12 after infection challenge. The DC transfer also enhanced cellular response to clear the infection to a great extent. Further, DC generated in the conditions with intact or deficient NK cell activity were analyzed for the efficiency to elicit protective immunity to C. muridarum infection (Fig. 5). These results indicate that NK–DC interactions after infection in vivo enable DC to acquire the functional ability to induce effective adaptive immune responses against chlamydial infection. In contrast, the adoptive transfer of NK DC failed to confer protection to recipient mice against C. muridarum infection. The Th1 cytokine production was greatly reduced in these mice. More specifically, mice transferred with NK-DC showed dramatic reduction in IFN-γ–producing CD4+ T cells compared with those with sham DC transfer after challenge infection. In support of these findings, we found that the functional capacity of DC isolated from NK cell-depleted mice was abrogated to polarize naïve CD4+ T cells to Th1 response in vitro. In addition, NK DC also showed decreased ability to induce IFN-γ production in C. muridarum-specific CD4+ T cells. Together, these findings demonstrated the physiological effect of NK cell interaction with DC in vivo in changing the functional response of DC to direct protective immune response to chlamydial infection challenge. The findings of the current study provide the first report, to our knowledge, on the effect of NK cells on DC function in immunity to chlamydial infection.

In conclusion, our findings presented here show that NK cells play a key role in the host defense against C. muridarum lung infection via modulating the function of DC. NK cells augmented the DC function to generate protective CD4+ cell response against chlamydial infection. These findings have important implications on the role of NK cells in shaping the adaptive immune response to infections and will support the idea of design of targeted therapies exploiting NK–DC interactions.

Disclosures
The authors have no financial conflicts of interest.

References


