Role of Natural Killer Cells in Modulating Dendritic Cell Responses to *Leishmania amazonensis* Infection[▽]

Mayra X. Hernandez Sanabria,¹ Diego A. Vargas-Inchaustegui,¹ Lijun Xin,¹ and Lynn Soong^{1,2*}

Department of Microbiology and Immunology¹ and Department of Pathology,² Center for Biodefense and Emerging Infectious Diseases, Sealy Center for Vaccine Development, Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, Texas

Received 9 April 2008/Returned for modification 13 May 2008/Accepted 1 September 2008

The importance of the interaction between natural killer (NK) cells and dendritic cells (DCs) in the expansion of antiviral and antitumor immune responses is well-documented; however, limited information on DC-NK cell interaction during parasitic infections is available. Given that some *Leishmania* parasites are known to prevent or suppress DC activation, we developed a DC-NK cell coculture system to examine the role of NK cells in modulating the functions of *Leishmania*-infected DCs. We found that the addition of freshly isolated, resting NK cells significantly promoted the activation of DCs that were preinfected with *Leishmania amazonensis* promastigotes and that these activated DCs, in turn, stimulated NK cell activation mostly via cell contact-dependent mechanisms. Notably, *L. amazonensis* amastigote infection failed to activate DCs, and this lack of DC activation could be partially reversed by the addition of preactivated NK (ANK) cells but not resting NK cells. Moreover, the adoptive transfer of ANK cells into *L. amazonensis*-infected mice markedly increased DC and T-cell activation and reduced tissue parasite loads at 1 and 3 weeks postinfection. These results suggest differential roles of DC-NK cell cross talk at different stages of *Leishmania* infection and provide new insight into the interplay of components of the innate immune system during parasitic infection.

Murine models of cutaneous leishmaniasis caused by *Leishmania major* infection have indicated the distinct roles of interleukin-4 (IL-4) production and IL-12-dependent gamma interferon (IFN- γ) production in disease susceptibility and resistance, respectively (33). However, following infection with *L. amazonensis* parasites in most inbred strains of mice, skin lesions progress without the evident expansion of IL-4-producing Th2 cells (38, 41). Recent in vitro and in vivo studies have highlighted the importance of dendritic cell (DC)-*Leishmania* interactions in priming and activating parasite-specific CD4⁺ T-cell subsets and shaping disease outcomes (32, 46). It becomes clear that the generation of long-lasting protective immunity against an infection requires coordinated interactions between innate and adaptive immune responses and between DCs and natural killer (NK) cells, two key components in the innate immune system (8, 31). The role of NK cells in protective immunity against infections with certain viruses, bacteria, or protozoa such as *Plasmodium falciparum* is well-documented (5, 6, 40). In murine models of *Leishmania* infection, NK cells contribute to the production of IFN- γ , a cytokine critical for activating leishmanicidal mechanisms in infected macrophages and activating Th1 cells (34), therefore promoting the control of *L. major* and *L. amazonensis* infections (25–27). Yet, limited information is available as to how NK cells are activated during *Leishmania* infection and how activated NK (ANK) cells contribute to the control of the infection.

Interactions between DCs and NK cells can result in cellular activation, maturation, and cytokine production by both cell types (13), and the supporting evidence was initially derived from in vitro studies of the DC-mediated activation of antitumor and antiviral effects in NK cells (3, 17). The findings of subsequent in vivo studies have indicated that DC-NK cell interactions occur primarily at the inflammation site and in draining lymph nodes (LNs) and that cytokines and chemokines produced by resident DCs and other cell types can attract immature DCs and NK cells to the initial infection site (11). Recently, Bajénoff and collaborators used adoptive cell transfer and a high-resolution imaging system to demonstrate interactions between DCs and NK cells in the draining LNs of mice infected with *L. major* (7). Following the uptake of live *Leishmania* parasites or parasite antigens at the infection site, DCs are activated to express high levels of major histocompatibility complex class II and costimulatory molecules (e.g., CD40 and CD80) and to produce proinflammatory cytokines (e.g., IL-12 and IL-1) (10, 30, 46). These DCs can also migrate to the draining LNs, where they interact with other cells such as NK and T cells. Thus, it is important to examine the details of DC-NK cell interaction in the context of infection with *Leishmania* parasites.

Using different species (*L. amazonensis*, *L. braziliensis*, and *L. major*) and developmental stages of parasites, as well as murine bone marrow-derived DCs (BM-DCs), we have recently demonstrated a species-dependent impairment in DC activation during infection with *L. amazonensis* promastigotes (42, 46) and a general lack of DC activation during infection with *L. amazonensis* amastigotes (37, 45). Given that DC activation can be enhanced by NK cells through cell contact-dependent and -independent mechanisms (14), we investigated whether NK cells would enhance DC responsiveness to *Leish-*

* Corresponding author. Mailing address: Department of Microbiology and Immunology, University of Texas Medical Branch, Medical Research Building 3.142, 301 University Blvd., Galveston, TX 77555-1070. Phone: (409) 772-8149. Fax: (409) 747-6869. E-mail: lysoong@utmb.edu.

[▽] Published ahead of print on 15 September 2008.

mania parasites. This report provides the first evidence of differential roles of resting NK and ANK cells in promastigote and amastigote infection in DCs and highlights the importance of DC-NK cell cross talk in innate immunity and host responses against *Leishmania* parasites.

MATERIALS AND METHODS

Mice. Female C57BL/6 (B6) and BALB/c mice and Syrian golden hamsters (Harlan Sprague Dawley, Indianapolis, IN), as well as IFN- γ -deficient B6 mice (Jackson Laboratory, Bar Harbor, ME), were used in this study. Animals were maintained under specific-pathogen-free conditions and used at 6 to 8 weeks of age, according to protocols approved by the institutional animal care and use committees.

Parasite cultures. The infectivity of *L. amazonensis* (strains MHOM/BR/77/LTB0016 and RAT/BA/74/LV78) was maintained by regular passage through BALB/c mice, and the infectivity of *L. braziliensis* (strain MHOM/PE/91/LC1418) was maintained by passage through hamsters. Promastigotes were cultured at 23°C in Schneider's *Drosophila* medium (Invitrogen, Carlsbad, CA), pH 7.0, supplemented with 20% fetal bovine serum (FBS; HyClone, Logan, UT), 2 mM L-glutamine, and 50 μ g of gentamicin/ml. Stationary-phase promastigote cultures passaged fewer than five times were used for DC or animal infection. Three sources of amastigotes were used in this study. Lesion-derived amastigotes (strain LTB) were obtained from foot lesions of BALB/c mice (infected with 2×10^6 promastigotes for 7 to 8 weeks) and cultured at 33°C in complete Grace's insect medium (Invitrogen), pH 5.0, containing 20% FBS, for 2 to 3 days prior to use. Axenic *L. amazonensis* amastigotes (strain LV78; obtained from Kwang-Poo Chang, Department of Microbiology and Immunology, Chicago Medical School) were maintained routinely in complete Grace's medium at 33°C (15). *L. braziliensis* amastigotes (LC1418) were freshly transformed from low-passage-number promastigotes and cultured in complete Grace's medium at 33°C and 5% CO₂ (42). To prepare heat-killed parasites, we incubated amastigotes (8×10^7 /ml in phosphate-buffered saline [PBS]) in a 60°C water bath for 15 min, as described previously (32).

DC generation. DCs were generated from B6 bone marrow in complete Iscove's modified Dulbecco's medium (Invitrogen) containing 10% FBS and supplemented with 20 ng of recombinant granulocyte-macrophage colony-stimulating factor (eBioscience, San Diego, CA)/ml or 6% culture supernatants of J558L cells that were stably transfected with the murine *gm-csf* gene (14). Non-adherent BM-DCs were harvested on day 8 and used in all experiments.

Preparation of resting NK and ANK cells. NK1.1⁺ cells were purified from the spleens of naïve B6 mice by negative selection using magnetic beads (Miltenyi Biotec, Auburn, CA), and their purity was routinely around 95%, as judged by flow cytometry. To generate ANK cells, purified NK1.1⁺ cells were cultured in complete Iscove's modified Dulbecco's medium supplemented with 4 ng of mouse recombinant IL-2 (BD Bioscience)/ml for 7 days. This protocol yielded 85% NK1.1⁺ CD69⁺ ANK cells, as described in a previous report (47).

DC infection and DC-NK cell cocultures. For DC infection studies, BM-DCs were seeded into 24-well plates (5×10^5 cells/well) and incubated with parasites (at a 2:1, 4:1, or 8:1 parasite-to-cell ratio) at 33°C for 12 h and then at 37°C for an additional 12 h. At an 8:1 parasite-to-DC ratio, DC infection rates were usually 50 to ~56% for *L. amazonensis* and *L. braziliensis* promastigotes and 68 to ~72% for *L. amazonensis* and *L. braziliensis* amastigotes, based upon fluorescence-activated cell sorter (FACS) analyses of carboxyfluorescein succinimidyl ester-labeled parasites (42, 46) (data not shown). Lipopolysaccharide (LPS; 100 ng/ml) from *Salmonella enterica* serovar Typhimurium (Sigma) was included in all experiments and used as a positive control. For DC-NK cell cocultures, DCs were infected with parasites for 4 or 24 h and then purified NK1.1⁺ resting NK cells or NK1.1⁺ CD69⁺ ANK cells were added to the culture at 2:1, 1:1, and 1:2 DC-to-NK cell ratios for an additional 18 h. Given that a 1:1 DC-to-NK cell ratio consistently gave the best results in pilot studies (judged on the DC activation status), we used this ratio in the rest of the experiments described in this study. To assess contact-dependent interactions, DCs and NK cells were cultured in transwell plates in compartments separated by a 0.4- μ m-pore-size polyester membrane (Becton Dickinson Labware, Franklin Lakes, NJ). After incubation, cells were collected for FACS analysis and the culture supernatants were harvested for cytokine detection.

Flow cytometry analysis. The following monoclonal antibodies and isotype controls were purchased from eBioscience: fluorescein isothiocyanate-conjugated anti-NK1.1 (PK136), anti-CD80 (16-10A1), anti-CD86 (GL1), and anti-CD4 (GK1.5); phycoerythrin (PE)-conjugated anti-CD40 (3/23), anti-CD83 (Michel-17), anti-CD69 (H1.2F3), and anti-CD62L (MEL-14); PE-Cy5-conju-

gated anti-CD11c (N418), anti-CD3e (145-2C11), and anti-CD44 (IM7); fluorescein isothiocyanate-conjugated rat immunoglobulin G2a (IgG2a); PE-conjugated rat IgG1, IgG2a, and IgG2b; and PE-Cy5-conjugated hamster IgG. The staining procedures were performed on ice. Briefly, cells were washed, blocked with 1 μ g of anti-mouse CD16/CD32 (eBioscience) per 10^6 cells, and then stained for specific surface molecules. Cells were acquired on a FACScan flow cytometer (BD Biosciences) and analyzed using FlowJo software version 8.5 (TreeStar, Ashland, OR).

Cytokine measurement. The levels of cytokines in culture supernatants were measured using enzyme-linked immunosorbent assays (ELISAs) and Bio-Plex assays. The detection limits for the IL-12p40 ELISA (BD Biosciences) and the IFN- γ ELISA (eBioscience) were 10 and 15 pg/ml, respectively. The custom-made mouse multiplex antibody bead kit (Invitrogen) allowed the detection of the following cytokines and chemokines: IL-1 α , IL-1 β , IL-2, IL-10, IL-12p40/p70, IL-17, CCL2/monocyte chemoattractant protein 1, CCL3/macrophage inflammatory protein 1 α , CCL5/RANTES, and CXCL10/IFN-inducible protein 10. The samples were analyzed on the Bio-Plex system powered by Luminex Xmap technology (Bio-Rad, Hercules, CA) using 5.0 software for a 4.1 workstation.

Mouse treatment and infection. B6 mice were infected subcutaneously (s.c.) in both feet with 2×10^5 stationary-phase *L. amazonensis* promastigotes. Mice (three per group) were treated with the following: intraperitoneal injection with PBS or recombinant mouse CXCL10 (100 ng/mouse; Leinco Technologies, St. Louis, MO) on the day of infection or s.c. injection in the foot with 3×10^5 ANK cells at 24 h postinfection. At 1 and 3 weeks postinfection, popliteal draining LN cells were collected and stained immediately for the expression of activation markers on DCs and NK and CD4⁺ cells. Footpad tissues were collected for parasite load analysis by real-time PCR.

Quantification of parasite loads by real-time PCR. Since the gene encoding *L. amazonensis* cysteine proteinase isoform 1 (*Llacys1*) is present as a single copy per haploid genome and is expressed in both the promastigote and amastigote stages, we selected the *Llacys1* gene for quantifying tissue parasite loads, as described previously (24). Total DNA was extracted from foot tissues or cell cultures by using a DNeasy blood and tissue kit (Qiagen, Valencia, CA), and 10 ng of DNA was used for parasite detection by real-time PCR at the University of Texas Medical Branch real-time PCR core facility (all reagents were purchased from Applied Biosystems, Foster City, CA). The sequences (5' to 3') for the amplification and detection of *Llacys1* included the forward (TCGTGCTGGG CCTTCTC) and reverse (TTGACGCCACTGACCTT) primers, as well as a 6-carboxyfluorescein dye-labeled capture probe (CCATTGGCAACATCG). The PCR cycling conditions were as follows: 40 cycles of uracil-N-glycosylase activation at 50°C for 2 min, AmpliTaq activation at 95°C for 10 min, denaturation at 95°C for 15 s, and annealing and extension at 60°C for 1 min on an ABI 7000 instrument (Applied Biosystems). Each sample was run in duplicate, and the results were normalized by the amount of total DNA extracted. The numbers of parasites per sample were then calculated based on a standard curve, which was generated for each PCR by using a DNA mixture that contained 10 ng of uninfected-mouse DNA and increasing amounts (0, 1, 10, 10², 10³, and 10⁴ pg) of *L. amazonensis* amastigote DNA. Our pilot studies indicated that 1 pg of amastigote DNA was equivalent to 12.4 parasites, while 10⁴ pg of amastigote DNA was equivalent to 1.2×10^5 parasites.

Statistical analysis. The difference between two different groups was determined by Student's *t* test. The tests were performed using GraphPad Prism, version 4.00, for Windows (GraphPad Software, San Diego, CA). *P* values indicating statistical significance were grouped into values of <0.05 and those of <0.01.

RESULTS

Cell contact-dependent activation of promastigote-infected DCs by NK cells. Our recent studies have indicated deficient DC activation during infection with *L. amazonensis* promastigotes (46). Given that DC activation can be enhanced by NK cells through cell contact-dependent and -independent mechanisms (14), we wondered whether the inclusion of NK cells could enhance the activation of *Leishmania*-infected DCs. To evaluate this possibility, we used a DC-NK cell coculture system in which DCs were preinfected with promastigotes or pretreated with LPS (a positive control) for 4 h and then freshly purified NK1.1⁺ cells were added to the culture at a 1:1 DC-to-NK cell ratio for an additional 18 h. The DC activation

FIG. 1. The coculture of *L. amazonensis* promastigote-infected DCs with resting NK cells enhanced DC and NK cell activation via direct cell contact. BM-DCs of B6 mice were seeded into 24-well plates (5×10^5 cells/well) and incubated with promastigotes (Pm; 8:1 parasite-to-cell ratio) or with LPS (100 ng/ml). After 4 h of infection, purified NK1.1⁺ cells were added to the cultures at a 1:1 DC-to-NK cell ratio for an additional 18 h. In some cases, NK cells and DCs were cultured in transwell plates in compartments separated by a 0.4-μm-pore-size polyester membrane. (A) Cells were collected and stained for a DC surface marker (CD11c) and activation markers (e.g., CD40). Data are presented as means $\pm$ standard deviations (SD) of percentages of CD11c⁺ CD40⁺ cells obtained from six independent experiments. The levels of IL-12p40 in the supernatants were determined by ELISA, and results are shown as means $\pm$ SD of values obtained from six independent experiments having similar results. (B) Cells were collected and stained for an NK cell surface marker (NK1.1) and an activation marker (CD69), and data are presented as means $\pm$ SD of percentages of NK1.1⁺ CD69⁺ cells obtained from six independent experiments. The levels of IFN-γ in the supernatants were determined by ELISA, and results are shown as the means $\pm$ SD of values obtained from six independent experiments. * ($P < 0.05$) and ** ($P < 0.01$) indicate statistically significant differences.

status was measured by the surface expression of CD40 on CD11c⁺ cells and the production of IL-12p40, two reliable parameters for DC activation during *Leishmania* infection in vitro (32, 46). As shown in Fig. 1A, while promastigote infection alone resulted in an increase in the percentage of CD11c⁺ CD40⁺ DCs (comparing <8% before infection to 20% postinfection; $P < 0.05$), the addition of NK cells led to a further increase (to 50%) in the percentage of CD11c⁺ CD40⁺ DCs ($P < 0.05$), as well as a significant increase in the production of IL-12p40 in culture supernatants ($P < 0.05$). To assess the biological relevance of this enhanced DC activation and IL-12p40 production, we measured the expression of CD69 on the surfaces of NK1.1⁺ cells and the levels of IFN-γ in culture supernatants. It was evident that the coculture of promastigote-infected DCs with resting NK cells also significantly promoted NK cell activation and IFN-γ production ($P < 0.05$) (Fig. 1B). IL-12p40 and IFN-γ were produced almost solely by activated DCs and NK cells, respectively (data not shown). This mutual activation of DC and NK cells was observed to be cell contact dependent, because of the significant reduction in CD11c⁺ CD40⁺ DCs and NK1.1⁺ CD69⁺ NK cells, as well as

FIG. 2. The coculture of *L. amazonensis* promastigote-infected DCs with resting NK cells enhanced DC and NK cell activation. BM-DCs were incubated with promastigotes (Pm; at an 8:1 parasite-to-cell ratio) or with LPS (100 ng/ml). After 24 h of infection, purified NK1.1⁺ cells were added to the culture at a 1:1 DC-to-NK cell ratio. After 18 h of coculture, cells were collected and stained for CD11c, CD40, NK1.1, and CD69, as described in the legend to Fig. 1. Shown are the means $\pm$ SD of percentages of CD11c⁺ CD40⁺ DCs (A) and NK1.1⁺ CD69⁺ NK cells (B) obtained from six independent experiments. The levels of IL-12p40 (A) and IFN-γ (B) in the supernatants were measured by ELISA. Results are shown as the means $\pm$ SD of values obtained from six independent experiments with similar results. * ($P < 0.05$) indicates statistically significant differences.

IL-12p40 and IFN-γ, under transwell conditions (Fig. 1). To confirm and extend these findings, we preinfected DCs for 24 h prior to the addition of purified NK1.1⁺ cells. As shown in Fig. 2A, while promastigote infection yielded less than 10% CD11c⁺ CD40⁺ DCs, the addition of NK cells resulted in an increase in the proportion of CD11c⁺ CD40⁺ DCs to more than 20% ($P < 0.05$). Likewise, the coculture of NK cells with preinfected DCs significantly promoted NK cell activation, as judged by increased CD69 expression and IFN-γ production ($P < 0.05$) (Fig. 2B). Together, these results suggest a mutual, cell contact-dependent activation of DCs and NK cells during infection with *L. amazonensis* promastigotes in vitro.

***L. amazonensis* amastigote infection failed to activate DCs even in the presence of NK cells.** Since *Leishmania* promastigotes interact with the mammalian host only briefly, we next examined the DC-NK cell interaction during infection with amastigotes, the disease-forming stage of the parasite. In this study, we used *L. amazonensis* amastigotes that were recently derived from either mouse lesions (strain LTB) or long-term cultures (strain LV78). DCs infected with amastigotes from either source showed no signs of activation, regardless of whether resting NK cells were added to DCs at 4 h postinfection (Fig. 3A). Likewise, there were no major signs of NK cell activation, except for an occasional increase in IFN-γ production in some groups (Fig. 3B). Similar trends were observed when DCs were preinfected with amastigotes for 24 h prior to the addition of NK cells (data not shown). These data confirmed our previous findings that infection alone with lesion-derived *L. amazonensis* amastigotes or amastigotes from long-

FIG. 3. Infection with *L. amazonensis* amastigotes failed to activate DCs, even in the presence of NK cells. (A and B) BM-DCs were exposed to amastigotes (Am; 8:1 parasite-to-cell ratio) from one of two sources: either a lesion-derived LTB strain or an axenically cultured LV78 strain. DCs treated with LPS (100 ng/ml) served as positive controls. After 4 h of infection, purified NK1.1⁺ cells were cocultured with DCs (at a 1:1 ratio). After 18 h of coculture, cells were collected and stained for CD11c, CD40, NK1.1, and CD69, as described in the legend to Fig. 1. Shown are the means $\pm$ SD of percentages of CD11c⁺ CD40⁺ DCs (A) and NK1.1⁺ CD69⁺ NK cells (B) obtained from three independent experiments with similar results. The levels of IL-12p40 (A) and IFN- γ (B) in the supernatants were measured by ELISA, and the results are shown as the means $\pm$ SD of values obtained from three independent experiments with similar results. (C) BM-DCs were incubated with either live or heat-killed LTB strains of amastigotes at 2:1, 4:1, and 8:1 parasite-to-cell ratios. DCs treated with LPS (100 ng/ml) served as positive controls. After 4 h of infection, purified NK1.1⁺ cells were added to the culture at a 1:1 DC-to-NK cell ratio. After 18 h of coculture, cells were collected and stained for DC surface markers (CD11c) and activation markers (e.g., CD40), and the results are presented as means $\pm$ SD of percentages of CD11c⁺ CD40⁺ DCs obtained from three independent experiments with similar results. Similar trends were observed when DCs were infected with live or heat-killed parasites for 24 h prior to the addition of NK cells (data not shown). * ($P < 0.05$) indicates statistically significant differences.

term cultures fails to activate DCs (45) and further suggested that the coculture of such infected DCs with resting NK cells fails to stimulate DC activation.

To verify whether the lack of DC activation was related to the infection dose or parasite replication, we used lesion-de-

FIG. 4. *L. braziliensis* amastigotes were capable of activating DCs. BM-DCs were incubated with *L. amazonensis* (La) and *L. braziliensis* (Lb) promastigotes (Pm) and amastigotes (Am) at an 8:1 ratio or with LPS (100 ng/ml). After 4 h of infection, purified NK1.1⁺ cells were cocultured with DCs (at a 1:1 ratio). After 18 h of coculture, cells were collected and stained for CD11c, CD40, NK1.1, and CD69, as described in the legend to Fig. 1. Results are presented as the percentages of CD11c⁺ CD40⁺ DCs (A) and NK1.1⁺ CD69⁺ NK cells (B), and the means $\pm$ SD of values obtained from three independent experiments are shown. * ($P < 0.05$) indicates statistically significant differences between the infected treated groups and the medium controls, while # ($P < 0.05$) indicates statistically significant differences between the selected groups. Dashed lines indicate the baseline levels for medium controls.

rived, live or heat-killed amastigotes (LTB) at different parasite-to-cell ratios (2:1, 4:1, and 8:1). It was evident that DCs infected with low doses or different preparations (live or heat killed) of amastigotes were not activated, even in the presence of NK cells (Fig. 3C). NK cell activation was not significant in any experimental group (data not shown). Similar trends were observed when NK cells were added to DCs at 24 h postinfection (data not shown).

***L. braziliensis* amastigotes were capable of activating DCs.** Because the infection with *L. amazonensis* amastigotes failed to induce DC and NK cell activation, we wanted to confirm whether this impairment was parasite species specific and performed similar studies using *L. braziliensis* amastigotes from short-term cultures (42). As shown in Fig. 4A, infection with *L. braziliensis* amastigotes alone resulted in significantly higher percentages of CD11c⁺ CD40⁺ DCs than that with *L. amazonensis* amastigotes (23 versus 13%; $P < 0.05$). Likewise, relatively high percentages of NK1.1⁺ CD69⁺ NK cells were detected after NK cells were treated with *L. braziliensis* amastigotes (Fig. 4B). However, DC-NK cell cocultures did not further enhance DC or NK cell activation. Nevertheless, comparative studies with two developmental stages of *L. amazonensis* and *L. braziliensis* parasites confirmed our previous findings (42) and suggested to us that the lack of infection-mediated DC and NK cell activation was unique to *L. amazonensis* amastigotes.

Effect of ANK cells in activating amastigote-infected DCs. Since the DC activation status can be influenced by ANK cells and T cells (12, 36), we asked whether preactivated NK cells

FIG. 5. Effect of ANK cells on the activation of *L. amazonensis*-infected DCs. BM-DCs were incubated with LTB promastigotes (Pm) or amastigotes (Am) at an 8:1 ratio or with LPS (100 ng/ml). After 4 h of infection, purified NK1.1⁺ cells and ANK cells were added to the culture at a 1:1 DC-to-NK cell ratio. After 18 h of coculture, cells were collected and stained for CD11c, CD40, NK1.1, and CD69, as described in the legend to Fig. 1. The results are presented as the percentages of CD11c⁺ CD40⁺ DCs (A) and NK1.1⁺ CD69⁺ NK cells (B), and the means $\pm$ SD of values obtained from three independent experiments with similar results are shown. * ($P < 0.05$) indicates statistically significant differences. La, *L. amazonensis*.

(ANK cells) could overcome immune deficiencies in *L. amazonensis* amastigote-infected DCs. We generated ANK cells via the cultivation of purified NK1.1⁺ cells with recombinant IL-2 for 7 days and added these cells to DCs at 4 h postinfection with amastigotes. In comparison to the addition of resting NK cells to DCs (Fig. 5A), the addition of ANK cells to *L. amazonensis* amastigote-infected DCs significantly enhanced the expression of CD40, CD80, and CD83, but not CD86, on CD11c⁺ DCs (Fig. 5A) ($P < 0.05$). The activation statuses of resting NK and ANK cells and the responsiveness of these NK cells to LPS and parasite infection alone are shown in Fig. 5B. Collectively, these FACS results suggest the potential of ANK cells, but not resting NK cells, for activating *L. amazonensis* amastigote-infected DCs.

We have previously shown that the early production of multiple inflammatory cytokines and chemokines is essential for the control of *L. major* infection and that the failure to induce these cytokines and chemokines is responsible for nonhealing lesions in *L. amazonensis*-infected mice (23). To further examine the biological relevance of DC-NK cell cocultures, we measured the levels of inflammatory cytokines and chemokines via ELISAs and Bio-Plex assays (the latter allow the detection of 11 molecules simultaneously). Consistently, we found that the coculture of activated but not resting NK cells and amastigote-infected DCs significantly promoted the production of IL-

FIG. 6. Production of IL-12p40 and IFN- γ in DC-NK cell cocultures. BM-DCs were incubated with *L. amazonensis* promastigotes (Pm) or amastigotes (Am) at an 8:1 ratio or with LPS (100 ng/ml). At 4 h postinfection, purified NK1.1⁺ cells or ANK cells were added to the culture at a 1:1 DC-to-NK cell ratio for an additional 18 h of incubation. The levels of IL-12p40 (A) and IFN- γ (B) in the culture supernatants were determined by specific ELISAs. Results are shown as the means $\pm$ SD of values obtained from three independent experiments with similar results. * ($P < 0.05$) and ** ($P < 0.01$) indicate statistically significant differences.

12p40 ($P < 0.05$) (Fig. 6A). Of note, there was an approximately 10-fold increase in IFN- γ production by amastigote-infected DCs cocultured with ANK cells compared to that by infected DCs cocultured with resting NK cells ($P < 0.01$) (Fig. 6B). Consistent with our data presented above (Fig. 1 and 2), significantly high levels of IL-12p40 and IFN- γ were detected in cocultures of promastigote-infected DCs and resting NK cells (Fig. 6). To assess the requirement for IFN- γ in DC activation and parasite control in this system, we generated ANK cells from IFN- γ ^{-/-} and wild-type B6 mice and cocultured these cells with amastigote- or promastigote-infected DCs. As illustrated in Fig. 7A, there were no major differences in DC activation levels (the percentages of CD11c⁺ CD40⁺ cells) when ANK cells were derived from wild-type or IFN- γ -deficient mice, although the differential roles of resting NK and ANK cells in promastigote and amastigote infection were still evident (Fig. 7B). Interestingly, we found that parasite loads in the IFN- γ -deficient ANK cell group were significantly higher than those in the wild-type counterparts at 18 h postinfection (Fig. 7C) ($P < 0.05$) and that the IFN- γ -deficient ANK cell group had twofold more parasites at 72 h postinfection (Fig. 7C). These results suggested that while IFN- γ production did not directly contribute to the activation of *L. amazonensis*-infected DCs, IFN- γ played an important role in the control of promastigote loads in DCs. With regard to other proinflammatory cytokines and chemokines, we detected a significant

FIG. 7. Role of IFN- γ in the activation of infected DCs and the control of parasite loads in DCs. BM-DCs were incubated with *L. amazonensis* (*La*) promastigotes (Pm) or amastigotes (Am) at an 8:1 ratio. At 4 h postinfection, resting NK cells and ANK cells derived from wild-type (wt) or IFN- γ ^{-/-} (knockout [ko]) mice were added to the culture (1:1 DC-to-NK cell ratio), and the cells were cocultured for an additional 18 or 72 h. Cells were collected and stained for a DC surface marker (CD11c) and activation markers, including CD40 (A), as well as CD80, CD83, and CD86 (data not shown). (A) Shown are representative plots from one of three experiments; the numbers in the upper right quadrants are the percentages. (B) The means $\pm$ SD of CD11c⁺CD40⁺ cells obtained from three independent experiments are presented in the graph. * ($P < 0.05$) indicates statistically significant differences between the infection control groups and coculture groups. (C) At 18 and 72 h postinfection, cells were collected to measure parasite loads by real-time PCR. Data are presented as mean *Lacys1* gene copy numbers (10^4) from three independent experiments. * ($P < 0.05$) and ** ($P < 0.01$) indicate statistically significant differences within groups, while # ($P < 0.05$) and ## ($P < 0.01$) indicate statistically significant differences between the selected groups.

increase in the production of IL-1 α , tumor necrosis factor alpha (TNF- α), IL-10, CCL3, and CXCL10 in cocultures of promastigote-infected DCs and resting NK cells but not in infected DCs alone (Fig. 8). Interestingly, cocultures of amastigote-infected DCs and ANK cells, but not resting NK cells, resulted in a significant increase in the production of TNF- α , CCL3, CCL5, and CXCL10 (Fig. 8) and IL-12p40/p70 (data not shown) compared to that in cocultures of DCs and resting NK cells. The increased production of these cytokines and chemokines appeared to be selective, because the production of CCL2 was partially reduced below the baselines (Fig. 8B) while the production of IL-2 and IL-1 β was unchanged (data not shown). Collectively, these data revealed differential roles of resting NK and ANK cells in modulating DC responses to *Leishmania* promastigotes and amastigotes.

Role of ANK cells in *L. amazonensis* infection in vivo. To confirm some of our in vitro findings, we further explored the in vivo function of ANK cells. B6 mice were infected s.c. with 2×10^5 promastigotes in the hind footpad and then injected s.c. with 3×10^5 ANK cells per foot at 24 h postinfection. Since injection with CXCL10 has been shown to enhance DC and NK cell activation (29) and to promote the control of *L. ama-*

zonensis infection (43), we included this treatment as a positive control. We found that draining LNs of mice receiving an adoptive transfer of ANK cells or CXCL10 injections exhibited significant increases in the percentages of CD40⁺, CD80⁺, and CD83⁺ CD11c⁺ DCs at 1 week (Fig. 9A) and 3 weeks (data not shown) postinfection compared to those of mice without ANK cell or CXCL10 treatment. Likewise, after 1 week of infection, LN cells of ANK cell- and CXCL10-treated mice showed higher percentages of activated (NK1.1⁺ CD69⁺) NK cells and CD4⁺ effector T cells (CD44^{high} CD62L^{low} CD4⁺ and CD69⁺ CD4⁺ cells) than cells of naïve or infected controls (Fig. 9B and C). Of relevance to these immunological changes was the significant reduction in tissue parasite loads at 1 and 3 weeks postinfection compared with those in the infection controls (Fig. 9D). Together, these in vivo results suggest the potential of ANK cells for promoting DC activation and effector-T-cell expansion during *L. amazonensis* infection in vivo.

DISCUSSION

Cooperation between DCs and NK cells plays a critical role in early defenses against cancer and viral infection (1, 3). Further, there is a report that DC activation can be enhanced by

FIG. 8. Production of proinflammatory cytokines and chemokines other than IFN- γ in DC-NK cell cocultures. BM-DCs were incubated with *L. amazonensis* promastigotes (Pm) or amastigotes (Am) at an 8:1 ratio or with LPS (100 ng/ml). At 4 h postinfection, purified NK1.1⁺ cells or ANK cells were added to the culture at a 1:1 DC-to-NK cell ratio for an additional 18 h of incubation. The levels of the indicated cytokines (A) and chemokines (B) in the culture supernatants were determined by a Bio-Plex assay. Results are shown as the means $\pm$ SD of values obtained from three independent experiments with similar results. * ($P < 0.05$) and ** ($P < 0.01$) indicate statistically significant differences.

NK cells through cellular contact, as well as through soluble factors (14). The role of DC-NK cell interaction in parasitic infection remains poorly understood, although some reports suggest the participation of NK cells in the control of *Leishmania* infection in humans and mice (2, 4, 22, 28, 34). For example, Bajénoff and collaborators have recently demonstrated that in mice infected with *L. major*, NK cells form a network in the LNs, where they interact with DCs, promoting DC production of cytokines that may influence adaptive immune responses (7). The purpose of this study was to investigate the cross talk between DCs and NK cells in *L. amazonensis* infection. The results of our in vitro studies with promastigotes indicated a cell contact-dependent, mutual activation of DCs and NK cells, regardless of whether DCs were preinfected for 4 or 24 h (Fig. 1 and 2). It has been reported previously that *Leishmania* promastigotes can activate NK cells through Toll-like receptor 2 (9) and Toll-like receptor 9 (35). Likewise, we found that *L. amazonensis* promastigotes could directly activate NK cells to produce low levels of IFN- γ (Fig. 1 and 2), although the molecular basis underlying this activation was not defined. In addition, our data suggest that while

IFN- γ production does not directly contribute to DC activation, IFN- γ plays an important role in controlling parasite infection in DCs (Fig. 7). These observations are relevant for understanding innate immunity to the invading parasites at an early stage of infection, since DC activation is important for the control of intracellular pathogens (21). Given that infection with *L. amazonensis* promastigotes alone induces relatively low levels of DC maturation and activation (16, 46), the interaction of promastigote-infected DCs with NK cells would be important for innate immunity and the generation of parasite-specific T-cell responses (44).

Since a lack of DC activation is a hallmark for *L. amazonensis* amastigote infection (45), we were particularly interested in examining whether DC responsiveness to amastigotes would be increased by NK cells. Using different amastigote species (*L. amazonensis* and *L. braziliensis*), strains (LTB and LV78), and preparations (consisting of lesion-derived amastigotes, amastigotes from long-term cultures, and live or heat-killed amastigotes), we confirmed our previous findings that while infection with *L. braziliensis* amastigotes alone is sufficient to activate murine DCs (42), infection with *L. amazonensis* amas-

FIG. 9. Role of ANK cells in *L. amazonensis* infection in vivo. B6 mice were infected s.c. in both feet with 2×10^5 stationary-phase promastigotes. Groups of three mice each were injected intraperitoneally with CXCL10 (100 ng/mouse) or PBS on the day of infection, while another group of mice was injected s.c. with 3×10^5 ANK cells in both feet at 24 h postinfection. (A to C) At 1 and 3 weeks postinfection, popliteal draining LNs were collected and immediately stained to determine the percentages of activated DCs (CD40⁺, CD80⁺, CD83⁺-positive CD11c⁺ cells) (A), ANK cells (CD69⁺-positive NK1.1⁺ cells) (B), and activated CD4⁺ T cells (CD44^{high} CD62L^{low} CD69⁺-positive CD4⁺ cells) (C). (D) At 1 and 3 weeks postinfection, footpad tissues were collected for the analysis of parasite loads by real-time PCR. The results shown in panels A and B are means $\pm$ SD of values obtained from three independent experiments, whereas the results shown in panels C and D are means $\pm$ SD of values obtained from two independent experiments. * ($P < 0.05$) and ** ($P < 0.01$) indicate statistically significant differences between infected treated groups and the infection controls, while # ($P < 0.05$) and ## ($P < 0.01$) indicate statistically significant differences between infected treated groups and the PBS controls.

tigotes triggers no or minimal DC activation (Fig. 3 and 4) (45). In the light of our previous findings that DCs infected with *L. amazonensis* amastigotes fail to induce CD40-dependent IL-12 production (32), the results of the present study provide additional evidence supporting the view that *L. amazonensis* amastigotes are peculiar for their incompetence in stimulating DC activation and cytokine production in comparison to the amastigotes of the other *Leishmania* species (45).

The most interesting and important finding in this study was that the interaction of ANK cells, but not that of resting NK cells, with *L. amazonensis* amastigote-infected DCs could partially overcome the deficiencies in DC activation in vitro (Fig. 5 to 8) and at early stages of infection in vivo (Fig. 9). The potential of ANK cells for promoting DC maturation and T-cell priming was noted previously in studies involving human and mouse cells (18, 19). Accordingly, the relevant question here is how ANK cells overcome deficiencies in *L. amazonensis* amastigote-infected DCs. Our results suggest several possible mechanisms. First, ANK cells can prompt amastigote-infected DCs to express CD40, CD80, and CD83 but not CD86 (Fig. 5), because the high levels of expression of these costimulatory molecules in *L. major*-infected DCs are important for priming parasite-specific T-cell responses (30). Second, ANK cells have a high capacity to produce IFN- γ , and adding IFN- γ -producing ANK cells to amastigote-infected DCs promotes the production of multiple proinflammatory cytokines and chemokines in vitro (Fig. 6 and 8). The adoptive transfer of ANK cells into *L. amazonensis*-infected susceptible mice enhanced DC and CD4⁺ T-cell activation and reduced tissue parasite burdens (Fig. 9). Given that ANK cells may have a direct role in parasite lysis and macrophage resistance to *L. amazonensis* infection (4) and that we observed a requirement for IFN- γ production from ANK cells for the control of parasite loads in

infected DCs (Fig. 7), it will be important to further examine the molecular basis underlying both ANK cell-mediated DC activation in vivo and reduction in tissue parasite loads.

The deficient production of multiple proinflammatory cytokines and chemokines is a hallmark of nonhealing lesions associated with *L. amazonensis* infection (23). We have proposed previously that these immune deficiencies are likely due to impaired DC activation (45) and have examined the potential of exogenous IL-1 β and CXCL10 for stimulating *L. amazonensis*-infected DCs (43, 46). In this study, we have described the roles of resting NK cells in promoting the production of IL-12, IFN- γ , IL-1 α , TNF- α , IL-10, CCL3, and CXCL10 by *L. amazonensis* promastigote-infected DCs, as well as the role of ANK cells in promoting the production of IL-12, IFN- γ , TNF- α , CCL3, CCL5, and CXCL10 by amastigote-infected DCs. Given that NK cells and NKT cells are the major sources of CXCL10 and that IL-12, IFN- γ , and CXCL10 form a positive loop, regulating NK and Th1 cell activities (39, 48), our data suggest that the availability of ANK cells and cross talk between ANK cells and *L. amazonensis* amastigote-infected DCs can activate effector CD4⁺ T cells to reduce tissue parasite loads.

Collectively, our results indicate that bidirectional DC-NK cell cross talk which can enhance both the activation of DC and NK cells and the production of proinflammatory cytokines and chemokines occurs in the context of *L. amazonensis* infection. Although the mechanisms or molecules involved in this DC-NK cell cross talk remain unclear, previous reports have suggested the participation of NKG2D in DC-NK cell responses to *Toxoplasma gondii* antigens (20) and the involvement of proteins of high-mobility group I in DC-ANK cell interaction (36). Another important finding in the present study is that while infection with *L. amazonensis* promastigotes

could trigger DC activation, the subsequent interaction with resting NK cells promoted the activation of promastigote-infected DCs. In sharp contrast, infection with *L. amazonensis* amastigotes did not lead to DC and NK cell activation. This immune defect appeared to be specific to this New World parasite species and could be partially overcome by the addition of ANK cells but not resting NK cells. This study provides a framework for a better understanding of the differential roles of NK cell subsets in innate immune responses to New World *Leishmania* parasites.

ACKNOWLEDGMENTS

This study was supported by NIH grant AI043003 (to L.S.) and by the James W. McLaughlin Fellowship Fund (to D.A.V.-I.).

We thank Johanna C. Sierra and Giovanni Suarez for technical help and Mardelle Susman for assisting in manuscript preparation.

REFERENCES

- Adam, C., S. King, T. Allgeier, H. Braumuller, C. Luking, J. Mysliwicz, A. Kriegeskorte, D. H. Busch, M. Rocken, and R. Mocikat. 2005. DC-NK cell cross-talk as a novel CD4⁺ T-cell-independent pathway for antitumor CTL induction. *Blood* **106**:338–344.
- Amprey, J. L., J. S. Im, S. J. Turco, H. W. Murray, P. A. Illarionov, G. S. Besra, S. A. Porcelli, and G. F. Spath. 2004. A subset of liver NK T cells is activated during *Leishmania donovani* infection by CD1d-bound lipophosphoglycan. *J. Exp. Med.* **200**:895–904.
- Andrews, D. M., A. A. Scalzo, W. M. Yokoyama, M. J. Smyth, and M. A. Gli-Esposti. 2003. Functional interactions between dendritic cells and NK cells during viral infection. *Nat. Immunol.* **4**:175–181.
- Aranha, F. C., U. Ribeiro, Jr., P. Basse, C. E. Corbett, and M. D. Laurenti. 2005. Interleukin-2-activated natural killer cells may have a direct role in the control of *Leishmania (Leishmania) amazonensis* promastigote and macrophage infection. *Scand. J. Immunol.* **62**:334–341.
- Arase, H., and L. L. Lanier. 2004. Specific recognition of virus-infected cells by paired NK receptors. *Rev. Med. Virol.* **14**:83–93.
- Artavanis-Tsakonas, K., K. Eleme, K. L. McQueen, N. W. Cheng, P. Parham, D. M. Davis, and E. M. Riley. 2003. Activation of a subset of human NK cells upon contact with *Plasmodium falciparum*-infected erythrocytes. *J. Immunol.* **171**:5396–5405.
- Bajénoff, M., B. Breart, A. Y. Huang, H. Qi, J. Cazareth, V. M. Braud, R. N. Germain, and N. Glaichenhaus. 2006. Natural killer cell behavior in lymph nodes revealed by static and real-time imaging. *J. Exp. Med.* **203**:619–631.
- Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* **392**:245–252.
- Becker, I., N. Salaiza, M. Aguirre, J. Delgado, N. Carrillo-Carrasco, L. G. Koheb, A. Ruiz, R. Caervantes, A. P. Torres, N. Cabrera, A. Gonzalez, C. Maldonado, and A. Isibasi. 2003. *Leishmania* lipophosphoglycan (LPG) activates NK cells through Toll-like receptor-2. *Mol. Biochem. Parasitol.* **130**:65–74.
- Brandonisio, O., R. Spinelli, and M. Pepe. 2004. Dendritic cells in *Leishmania* infection. *Microbes Infect.* **6**:1402–1409.
- Buentke, E., L. C. Heffler, J. L. Wilson, R. P. Wallin, C. Lofman, B. J. Chambers, H. G. Ljunggren, and A. Scheynius. 2002. Natural killer and dendritic cell contact in lesional atopic dermatitis skin—Malassezia-influenced cell interaction. *J. Invest. Dermatol.* **119**:850–857.
- Campos-Martin, Y., M. Colmenares, B. Gozalbo-Lopez, M. Lopez-Nunez, P. B. Savage, and E. Martinez-Naves. 2006. Immature human dendritic cells infected with *Leishmania infantum* are resistant to NK-mediated cytotoxicity but are efficiently recognized by NKT cells. *J. Immunol.* **176**:6172–6179.
- Cooper, M. A., T. A. Fehniger, A. Fuchs, M. Colonna, and M. A. Caligiuri. 2004. NK cell and DC interactions. *Trends Immunol.* **25**:47–52.
- Degli-Esposti, M. A., and M. J. Smyth. 2005. Close encounters of different kinds: dendritic cells and NK cells take centre stage. *Nat. Rev. Immunol.* **5**:112–124.
- Dutta, S., D. Ray, B. K. Kolli, and K. P. Chang. 2005. Photodynamic sensitization of *Leishmania amazonensis* in both extracellular and intracellular stages with aluminum phthalocyanine chloride for photolysis in vitro. *Antimicrob. Agents Chemother.* **49**:4474–4484.
- Favali, C., N. Tavares, J. Clarendio, A. Barral, M. Barral-Netto, and C. Brodsky. 2007. *Leishmania amazonensis* infection impairs differentiation and function of human dendritic cells. *J. Leukoc. Biol.* **82**:1401–1406.
- Fernandez, N. C., A. Lozier, C. Flament, P. Ricciardi-Castagnoli, D. Bellet, M. Suter, M. Perricaudet, T. Tursz, E. Maraskovsky, and L. Zitvogel. 1999. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses *in vivo*. *Nat. Med.* **5**:405–411.
- Fujii, S., K. Liu, C. Smith, A. J. Bonito, and R. M. Steinman. 2004. The linkage of innate to adaptive immunity via maturing dendritic cells *in vivo* requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *J. Exp. Med.* **199**:1607–1618.
- Gerosa, F., B. Baldani-Guerra, C. Nisii, V. Marchesini, G. Carra, and G. Trinchieri. 2002. Reciprocal activating interaction between natural killer cells and dendritic cells. *J. Exp. Med.* **195**:327–333.
- Guan, H., M. Moretto, D. J. Bzik, J. Gligley, and I. A. Khan. 2007. NK cells enhance dendritic cell response against parasite antigens via NKG2D pathway. *J. Immunol.* **179**:590–596.
- Habib, M., M. N. Rivas, M. Chamekh, S. Wiekowski, W. Sun, A. Bianco, N. Trouche, O. Chaloïn, H. Dumortier, M. Goldman, G. Guichard, S. Fournel, and B. Vray. 2007. Cutting edge: small molecule CD40 ligand mimetics promote control of parasitemia and enhance T cells producing IFN- γ during experimental *Trypanosoma cruzi* infection. *J. Immunol.* **178**:6700–6704.
- Ishikawa, H., H. Hisaeda, M. Taniguchi, T. Nakayama, T. Sakai, Y. Maekawa, Y. Nakano, M. Zhang, T. Zhang, M. Nishitani, M. Takashima, and K. Himeno. 2000. CD4⁺ α 14 NKT cells play a crucial role in an early stage of protective immunity against infection with *Leishmania major*. *Int. Immunol.* **12**:1267–1274.
- Ji, J., J. Sun, and L. Soong. 2003. Impaired expression of inflammatory cytokines and chemokines at early stages of infection with *Leishmania amazonensis*. *Infect. Immun.* **71**:4278–4288.
- Lasakosvitch, F., L. G. Gentil, M. R. dos Santos, J. F. da Silveira, and C. L. Barbieri. 2003. Cloning and characterisation of a cysteine proteinase gene expressed in amastigotes of *Leishmania (L.) amazonensis*. *Int. J. Parasitol.* **33**:445–454.
- Laskay, T., A. Diefenbach, M. Rollinghoff, and W. Solbach. 1995. Early parasite containment is decisive for resistance to *Leishmania major* infection. *Eur. J. Immunol.* **25**:2220–2227.
- Laskay, T., M. Rollinghoff, and W. Solbach. 1993. Natural killer cells participate in the early defense against *Leishmania major* infection in mice. *Eur. J. Immunol.* **23**:2237–2241.
- Laurenti, M. D., M. Gidlund, D. M. Ura, I. L. Sinhorini, C. E. Corbett, and H. Goto. 1999. The role of natural killer cells in the early period of infection in murine cutaneous leishmaniasis. *Braz. J. Med. Biol. Res.* **32**:323–325.
- Maasho, K., F. Sanchez, E. Schurr, A. Hailu, and H. Akuffo. 1998. Indications of the protective role of natural killer cells in human cutaneous leishmaniasis in an area of endemicity. *Infect. Immun.* **66**:2698–2704.
- Mohan, K., E. Cordeiro, M. Vaci, C. McMaster, and T. B. Issekutz. 2005. CXCR3 is required for migration to dermal inflammation by normal and *in vivo* activated T cells: differential requirements by CD4 and CD8 memory subsets. *Eur. J. Immunol.* **35**:1702–1711.
- Moll, H. 2003. Dendritic cells and host resistance to infection. *Cell. Microbiol.* **5**:493–500.
- Moretta, A. 2002. Natural killer cells and dendritic cells: rendezvous in abused tissues. *Nat. Rev. Immunol.* **2**:957–964.
- Qi, H., V. Popov, and L. Soong. 2001. *Leishmania amazonensis*-dendritic cell interactions *in vitro* and the priming of parasite-specific CD4⁺ T cells *in vivo*. *J. Immunol.* **167**:4534–4542.
- Sacks, D., and N. Noben-Trauth. 2002. The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat. Rev. Immunol.* **2**:845–858.
- Scharton, T. M., and P. Scott. 1993. Natural killer cells are a source of interferon gamma that drives differentiation of CD4⁺ T cell subsets and induces early resistance to *Leishmania major* in mice. *J. Exp. Med.* **178**:567–577.
- Schleicher, U., J. Liese, I. Knippertz, C. Kurzmann, A. Hesse, A. Heit, J. A. A. Fischer, S. Weiss, U. Kalinke, S. Kunz, and C. Bogdan. 2007. NK cell activation in visceral leishmaniasis requires TLR9, myeloid DCs, and IL-12, but is independent of plasmacytoid DCs. *J. Exp. Med.* **204**:893–906.
- Semino, C., G. Angelini, A. Poggi, and A. Rubartelli. 2005. NK/iDC interaction results in IL-18 secretion by DCs at the synaptic cleft followed by NK cell activation and release of the DC maturation factor HMGB1. *Blood* **106**:609–616.
- Soong, L. 2008. Modulation of dendritic cell function by *Leishmania* parasites. *J. Immunol.* **180**:4355–4360.
- Soong, L., C. H. Chang, J. Sun, B. J. Longley, Jr., N. H. Ruddle, R. A. Flavell, and D. McMahon-Pratt. 1997. Role of CD4⁺ T cells in pathogenesis associated with *Leishmania amazonensis* infection. *J. Immunol.* **158**:5374–5383.
- Udai, P. S., S. Shailesh, S. Rajesh, C. Yingzi, D. T. Dennis, and W. L. James. 2008. CXCL10-producing mucosal CD4⁺ T cells, NK cells, and NKT cells are associated with chronic colitis in IL-10^{-/-} mice, which can be abrogated by anti-CXCL10 antibody inhibition. *J. Interferon Cytokine Res.* **28**:31–43.
- Vankayalapati, R., A. Garg, A. Porgador, D. E. Griffith, P. Klucar, H. Safi, W. M. Girard, D. Cosman, T. Spies, and P. F. Barnes. 2005. Role of NK cell-activating receptors and their ligands in the lysis of mononuclear phagocytes infected with an intracellular bacterium. *J. Immunol.* **175**:4611–4617.
- Vanloubbeek, Y. F., A. E. Ramer, F. Jie, and D. E. Jones. 2004. CD4⁺ Th1 cells induced by dendritic cell-based immunotherapy in mice chronically infected with *Leishmania amazonensis* do not promote healing. *Infect. Immun.* **72**:4455–4463.
- Vargas-Inchaustegui, D. A., L. Xin, and L. Soong. 2008. *Leishmania bra-*

- ziliensis* infection induces dendritic cell activation, ISG15 transcription, and the generation of protective immune responses. *J. Immunol.* **180**:7537–7545.
43. **Vasquez, R. E., and L. Soong.** 2006. CXCL10/gamma interferon-inducible protein 10-mediated protection against *Leishmania amazonensis* infection in mice. *Infect. Immun.* **74**:6769–6777.
44. **Walzer, T., M. Dalod, S. H. Robbins, L. Zitvogel, and E. Vivier.** 2005. Natural-killer cells and dendritic cells: “l’union fait la force.” *Blood* **106**: 2252–2258.
45. **Xin, L., K. Li, and L. Soong.** 2008. Down-regulation of dendritic cell signaling pathways by *Leishmania amazonensis* amastigotes. *Mol. Immunol.* **45**: 3371–3382.
46. **Xin, L., Y. Li, and L. Soong.** 2007. Role of interleukin-1 β in activating the CD11c^{high} CD45RB⁺ dendritic cell subset and priming *Leishmania amazonensis*-specific CD4⁺ T cells in vitro and in vivo. *Infect. Immun.* **75**:5018–5026.
47. **Xu, D., H. Liu, M. Komai-Koma, C. Campbell, C. McSharry, J. Alexander, and F. Y. Liew.** 2003. CD4⁺CD25⁺ regulatory T cells suppress differentiation and functions of Th1 and Th2 cells, *Leishmania major* infection, and colitis in mice. *J. Immunol.* **170**:394–399.
48. **Zaph, C., and P. Scott.** 2003. Interleukin-12 regulates chemokine gene expression during the early immune response to *Leishmania major*. *Infect. Immun.* **71**:1587–1589.

Editor: J. F. Urban, Jr.