Vaccination with Live *Leishmania major* and CpG DNA Promotes Interleukin-2 Production by Dermal Dendritic Cells and NK Cell Activation

Eva Maria Laabs,† Wenhu Wu, and Susana Mendez*

*James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853*

Received 19 June 2009/Returned for modification 30 July 2009/Accepted 16 September 2009

Cutaneous leishmaniasis due to *Leishmania major* is an emerging, chronic parasitic disease that causes disfigurement and social stigmatization. Drug therapy is inadequate, and there is no vaccine. Inoculation of virulent parasites (leishmanization) is the only intervention that has ever provided protection, because it mimics natural infection and immunity, but it was discontinued due to safety concerns (uncontrolled vaccinal lesions). In an effort to retain the benefits (immunity) while avoiding the side effects (lesions) of leishmanization, we immunized C57BL/6 mice with *L. major* and CpG DNA (Lm/CpG). This combination prevented lesions while inducing immunity. Also, the vaccination with live parasites and the Toll-like receptor 9 agonist enhanced innate immune responses by activating dermal dendritic cells (DCs) to produce cytokines. Here we report that the Lm/CpG vaccine induced dermal DCs, but not bone marrow-derived DCs, to produce interleukin-2 (IL-2). The release of this unusual DC-derived cytokine was concomitant with a peak in numbers of NK cells that produced gamma interferon (IFN-γ) and also enhanced activation of proliferation of IFN-γ+ CD4+ T cells. Parasite growth was controlled in Lm/CpG-vaccinated animals. This is the first demonstration of the ability of dermal DCs to produce IL-2 and of the activation of NK cells by vaccination in the context of leishmaniasis. Understanding how the Lm/CpG vaccine enhances innate immunity may provide new tools to develop vaccines against *L. major*, other chronic infectious diseases, or other conditions, such as cancer.

The leishmaniases are among the most important emerging parasitic diseases, second only to malaria in terms of the number of affected people. The prevalence of cutaneous leishmaniasis due to *Leishmania major*, a chronic disease leading to disfigurement, functional impairment, and social stigmatization, is estimated to be 2 million cases (4); recent data demonstrate that this number is greatly underestimated (1). Naïve individuals are very susceptible, leading to dramatic outbreaks. Current drugs are inadequate due to toxicity, resistance, cost, and adverse effects, and there is no vaccine. Thus, there is a clear need for both prophylactic and therapeutic control measures. Inoculation of virulent *L. major* (leishmanization), practiced in areas of leishmaniasis endemicity for more than 1,000 years, is the only strategy that has ever provided lifelong protection, because it mimics the natural infection, enabling parasite persistence and the subsequent concomitant immunity. It was widely applied but was discontinued due to the development of large vaccinal lesions in about 10% of the immunized patients (14).

Because no vaccine other than leishmanization has been successful with humans, we work to understand its mechanism of action. In an effort to retain the benefits (immunity) while avoiding the side effects (lesions) of leishmanization, we immunized C57BL/6 mice with *L. major* alone or in combination with CpG DNA (Lm/CpG). Lm/CpG prevented vaccinal lesions while achieving *L. major* persistence and immunity (13, 29). Mechanistically, we found that Lm/CpG causes early activation of dermal dendritic cells (DCs) to produce interleukin-6 (IL-6), reducing the accumulation of regulatory T cells (Tregs) in the vaccination site (29). Activated DCs also produced IL-12, promoting activation and proliferation of gamma interferon (IFN-γ)-producing CD4+ T cells (13, 29). The lack of suppressors and the increase in effectors resulted in parasite killing and a lack of vaccinal lesions. Interestingly, Treg numbers recovered in the skin of mice vaccinated with Lm/CpG, enabling concomitant immunity and lifelong protection (13), as previously demonstrated by us (3).

While continuing the study of the mechanism of action of the Lm/CpG vaccine, we detected a rapid increase in IL-2 expression in the vaccination site. IL-2 is a survival factor for B and T lymphocytes, but it is also necessary for DC-dependent NK cell activation (7). Thus, NK cells may be critical in the early control of parasite growth in the vaccinated mice. In this paper, we report the unusual IL-2 production by CD11c+ DCs in the skin of mice vaccinated with Lm/CpG. This unusual secretion is concomitant with a peak in IFN-γ-producing NK cells and CD4+ T cells and the control of parasite growth. Thus, vaccination with live parasites and Toll-like receptor 9 agonists appears to enhance not only adaptive immunity but also the response of innate immune cells to decrease parasite growth and the development of vaccinal pathology.

**MATERIALS AND METHODS**

*Mice.* C57BL/6 mice were purchased from Taconic (Germantown, NY). All mice were maintained in the Baker Institute animal care facility under pathogen-

---
free conditions. All animal experiments were performed according to federal guidelines and institutional policies.

**Infection protocol and vaccine preparation.** *L. major* clone V1 (MHOM/IL/80/Friedlin) promastigotes were grown at 26°C in medium 199 supplemented with 20% heat-inactivated fetal calf serum (Gemini, Sacramento, CA), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 40 mM HEPES, 0.1 mM adenine (in 50 mM HEPES), and 5 mg/ml hemin in 50% triethanolamine. Infective-stage promastigotes (metacyclics) of *L. major* were isolated from stationary cultures (4 to 5 days old) by Ficoll enrichment, as described previously (25). Mice were vaccinated intradermally in the ear with 10⁵ *L. major* metacyclic promastigotes alone or in combination with 50 μg CpG DNA (25 g Bacto beef, 500 ml water, 10 g neopeptone, 10 g Bacto agar, and 2.5 g NaCl) containing 20% of defibrinated rabbit blood overlaid with 100 μl NNN medium (Novy-MacNeal-Nicolle biphasic medium [25 g Bacto beef, 500 ml water, 10 g neopeptone, 10 g Bacto agar, and 2.5 g NaCl]) containing 20% of defibrinated rabbit blood overlaid with 100 μl of 100 U/ml penicillin-100 μg/ml streptomycin and Liberase CI enzyme blend to obtain single-cell suspensions, as described above.

**Analysis of dermal lymphocytes.** Ears were collected at different time points. The ventral and dorsal sheets of the ears were separated and deposited in RPMI containing 100 U/ml penicillin-100 μg/ml streptomycin and Liberase CI enzyme blend (0.5 mg/ml; Roche, Nutley, NJ). Ears were incubated for 60 min at 37°C. The sheets were dissociated using a handheld tissue homogenizer. The homogenates were diluted in a 96-well, flat-bottomed microtiter plate containing biphasic medium filtered using a 70-μm cell strainer (BD Falcon, San Jose, CA) and serially diluted using a 27.5-gauge needle in a volume of 10 μl.

**Determination of parasite burden in ears.** Briefly, the ventral and dorsal sheets of the vaccinated ears were separated and deposited in RPMI containing 100 U/ml penicillin-100 μg/ml streptomycin and Liberase CI enzyme blend (0.5 mg/ml; Roche, Nutley, NJ). Ears were incubated for 60 min at 37°C. The sheets were dissociated using a handheld tissue homogenizer. The homogenates were diluted in a 96-well, flat-bottomed microtiter plate containing biphasic medium prepared using 50 μl NNN medium (Noy-MacNeal-Nicolle biphasic medium [25 g Bacto beef, 500 ml water, 10 g neopeptone, 10 g Bacto agar, and 2.5 g NaCl]) containing 20% of defibrinated rabbit blood overlaid with 100 μl medium 199. The number of viable parasites in each ear was estimated by use of a 27.5-gauge needle in a volume of 10 μl.

**RESULTS**

**IL-2 secretion increases in the skin of mice vaccinated with live *L. major* and CpG DNA.** Our previous work has shown that vaccination with live parasites and CpG DNA affects the onset of the natural immune response of mice to *L. major*. Specifically, it causes early release of IL-6 and IFN-γ, concomitant with a decrease in IL-10 production (13, 29). We vaccinated mice with live *L. major* in the presence or absence of CpG DNA. As reported previously (29), mice vaccinated with Lm/CpG displayed significantly smaller lesions than animals vaccinated with *L. major* alone (Fig. 1). Using ELISA, we determined the kinetics of IL-2 secretion in the skin of the vaccinated mice. We found that IL-2 secretion was low in the vaccination site of *L. major*-inoculated mice at week 2 (400 pg/ml). In contrast, IL-2 secretion was significantly increased in the mice inoculated with Lm/CpG (3,200 pg/ml) at the same time point (Fig. 2). At 5 weeks postinfection, this pattern was reversed, with the *L. major*-vaccinated mice showing the greatest level of IL-2 production (4,500 pg/ml), even though IL-2 levels were still elevated in Lm/CpG-vaccinated animals (2,900 pg/ml) (*P* = 0.04). In both groups, IL-2 expression decreased at week 10, although it remained higher in the *L. major*-vaccinated group (*P* = 0.002). These data revealed that Lm/CpG vaccination induces early production of IL-2 in the skin of the vaccinated mice.

**CD11c⁺ cells are the main producer of IL-2 in Lm/CpG-vaccinated animals.** Because we have reported that CD4⁺ T cells accumulate at the site of Lm/CpG injection 2 weeks after vaccination (13), we aimed to investigate whether these lym-
Phocytes were the cells responsible for the increase in IL-2 levels. We performed a kinetic study in which IL-2 expression was investigated for B220<sup>+</sup> cells (B cells), CD8<sup>+</sup> T cells, CD4<sup>+</sup> T cells, F4/80<sup>+</sup> cells (macrophages), and CD11c<sup>+</sup> cells (DCs). Expression of the cytokine was determined by flow cytometry prior to vaccination and at weeks 1, 2, 3, 6, and 10 postvaccination in both *L. major*- and Lm/CpG-vaccinated animals. B cells did not express significant levels of IL-2 for the duration of the study (Fig. 3A). IL-2 expression was increased significantly (*P* < 0.0001) in CD8<sup>+</sup> T cells of mice vaccinated with *L. major* at week 5 postvaccination; the expression of the cytokine was only slightly significantly (*P* = 0.001) elevated in Lm/CpG-vaccinated mice at week 3 (Fig. 3B). The analysis of IL-2 production by CD4<sup>+</sup> T cells revealed a peak of IL-2 expression at week 2 in mice vaccinated with Lm/CpG (*P* = 0.0001), suggesting that a population of activated CD4<sup>+</sup> cells migrated to the site of Lm/CpG inoculation early after vaccination. This is consistent with our previously published data, where we reported an increase in activated, IFN-γ-producing CD4<sup>+</sup> T cells in the skin of Lm/CpG-vaccinated mice (13), likely to have migrated in response to vaccine-induced activated DCs. On the other hand, CD4<sup>+</sup> T cells from mice vaccinated with *L. major* expressed much higher levels of the cytokine (sixfold increase, *P* = 0.00001) at week 5 (Fig. 3C), indicating that T lymphocytes, and in particular CD4<sup>+</sup> T cells, are the major source of IL-2 in these mice. F4/80<sup>+</sup> macrophages did not express IL-2 (Fig. 3D). Finally, we observed that IL-2 expression in CD11c<sup>+</sup> DCs increased significantly from week 2 (*P* = 0.001) to week 3.
found that the numbers of NK cells (defined for C57BL/6 mice
in the ears of mice vaccinated with L. major promastigotes (Lm), 50 μg CpG DNA, or a combination of both and collected 24 h after stimulation to be analyzed by flow cytometry for IL-2 expression. Flow cytometry plots are representative of three independent experiments. Values indicate the percentages of CD11c+ cells expressing the cytokine. FSC, forward scatter.

\[ P = 0.008 \] in the skin of Lm/CpG-vaccinated animals (Fig. 3E). Interestingly, the number of CD11c+ DCs expressing IL-2 was even greater than the number of CD4+ T cells (15,000 versus 12,000; \( P = 0.0001 \)) in these animals, becoming the major source of IL-2 in the skin of Lm/CpG-inoculated mice. L. major-vaccinated animals showed only a modest increase in IL-2 expression by CD11c+ DCs.

**Dermal DCs, but not bone marrow-derived DCs, produce IL-2 in response to Lm/CpG vaccination.** Dermal DCs and bone marrow-derived DCs were obtained, seeded, and primed with L. major or Lm/CpG in vitro for 24 h, when cells were collected and analyzed for CD11c and IL-2 expression by flow cytometry. Figure 4 shows that dermal DCs primed with L. major alone did not induce any significant IL-2 expression. Priming of dermal DCs with CpG DNA alone induced a moderate increase in IL-2 expression (18% of cells expressed IL-2) compared to L. major-infected DCs. In contrast, CD11c+ cells from the skin of mice vaccinated with Lm/CpG significantly increased the expression of IL-2 (to 32% of cells) in response to the vaccine. Interestingly, bone marrow-derived DCs failed to express IL-2 when exposed to the vaccines in vitro. CpG DNA induced only a modest increase in IL-2 expression (to 8% of cells). These data indicate that dermal DCs (or a CD11c+ subset of this population), as opposed to bone marrow-derived DCs, have the ability to express IL-2 in response to Lm/CpG. Although CpG DNA induced the expression of the cytokine by itself, this phenomenon is enhanced by the coinoculation of L. major parasites.

**IFN-γ-producing NK cell numbers increase after vaccination with Lm/CpG in the skin at the time of parasite growth control.** IL-2 is a growth factor for T and B cells, acts on the differentiation of monocytes, and is necessary for the activation of NK cells. Our previous work has already established that Lm/CpG vaccination increased the frequency of CD4+ T cells and DCs at the site of inoculation (13, 29). Because NK cells have been implicated to participate in the control of leishmanization at the early stages of infection, we aimed to investigate the kinetics of NK cell migration and activation in dermal tissue in the ears of mice vaccinated with L. major or Lm/CpG. We found that the numbers of NK cells (defined for C57BL/6 mice as NK1.1+, CD3+ lymphocytes) increased during the first 3 weeks after vaccination (Fig. 5A), peaking at week 2, which coincided with the peak of IL-2 production found after vaccination with Lm/CpG (Fig. 1). The absolute number of IFN-γ+ NK cells (Fig. 5B) was also increased in those mice at the same time points, indicating that these cells were activated at the site of Lm/CpG vaccination. Figure 5C illustrates the increase in frequency of NK1.1+ CD3+ cells at 2 weeks postvaccination with Lm/CpG (to 10%), as well as the increase in IFN-γ expression (to 32%) in this population. This experiment was repeated four times, and the average frequencies of NK cells as well as the percentages of these cells producing IFN-γ are shown in Fig. 5D. In all cases, activated NK cell frequencies were significantly higher for the Lm/CpG-vaccinated group than for the L. major-vaccinated group at the 2-week time point. Finally, Fig. 5E represents the parasite burdens in the ears of L. major- and Lm/CpG-vaccinated animals at different time points. These results demonstrate that parasite growth is controlled in the latter group, as opposed to the former group, which shows a peak in parasite numbers at week 5.

**DISCUSSION**

Our recent work has focused on unraveling the immune features of leishmanization, because to date this has been the only effective strategy to prevent disease caused by L. major in humans. By understanding the components of the immune response to live vaccination that are responsible for immunity and protection, we aim to apply them to the development of safer killed or subunit vaccines.

Our approach to leishmanization (inoculation of live parasites with CpG DNA) (3, 13) has proven safer than the original live vaccine (L. major alone) because mice vaccinated with Lm/CpG do not develop lesions caused by infection. The study of the mechanism of action has revealed profound differences in both the kinetics and the quality of the immune response in Lm/CpG- versus L. major-vaccinated animals. We have discovered that the Lm/CpG vaccine causes early dermal DC activation (29), which leads to increased IL-6 and IL-12 production. This in turn causes the early migration of IFN-γ+ CD4+ T cells (13) and a decrease in Treg numbers in the vaccination site (29). These two events result in the rapid elimination of the parasites present in the vaccine and no pathology. Tregs, however, are able to successfully establish in the chronic site of Lm/CpG vaccination, enabling long-term immunity (3, 13).

In this report, we have also discovered that the Lm/CpG...
vaccine enhances IL-2 production by dermal DCs and not other antigen-presenting cells. For the mice vaccinated with the Lm/CpG vaccine, CD11c^+ DCs become the major source of the cytokine for a sustained period of 2 to 5 weeks. We hypothesize that this may have two main consequences: reestablishment of Tregs in the vaccination site (Tregs are highly dependent on IL-2 for their survival [28]) and enhancement of lymphocyte activation, especially in NK cells and CD4^+ Th1 cells. Although immunity to L. major in resistant strains, such as C57BL/6, is conferred mainly by the generation of a Th1 response (19), a number of studies have investigated the role of innate cells in protection against the parasite. Whereas NK T cells may play a role in modulating visceralization of Leishmania parasites (12, 26), it has been proposed that early IFN-γ produced by NK cells is important in the regulation of CD4^+ T-cell subset development and early resistance to L. major (10,
The exact contribution of NK cells to the control of L. major infection remains controversial (20), although it is clear that increased NK cell activity would result in parasite killing and development of CD4+ T-cell effectors (10, 21).

The analysis of the requirements for NK cell activation in resistant mice has revealed that the NK cell response may be IL-2 and IL-12 dependent (22). The role of IL-12 in the activation of NK cells in leishmaniasis, however, remains undefined: it has been shown that NK cells are activated by CpG DNA via autocrine DC-derived IL-12 (17, 22, 24, 27), but other authors have demonstrated that neutralization of IL-12 enhanced NK cell activity (10, 18, 23). In contrast, a role for IL-2-derived DCs in the activation of NK cells has been demonstrated in vitro and in vivo (5–9, 15, 16). Because NK cells do not express Toll-like receptor 9 (2), it appears that the most likely mechanism is that CpG DNA acts on DCs to enhance IL-2 production and activate DCs, as has been suggested in other experimental models (8, 30).

To date, no vaccine against leishmaniasis has investigated the repercussions of vaccination on NK cells. Further investigations of this newly recognized effect (enhancement of NK function by the Lm/CpG vaccine) need to be conducted to understand (i) the exact population(s) that produces IL-2 and (ii) the specific role of each vaccine component (parasites and CpG DNA). The understanding of the mechanism of action of the Lm/CpG vaccine and its effect on NK cell activation could also be exploited for therapeutic purposes (e.g., cancer vaccines). Furthermore, it would be useful to design killed or subunit antileishmanial vaccines that could reproduce such an effect.

ACKNOWLEDGMENT

This work was supported by the National Institute of Allergy and Infectious Diseases, NIH, through research grant R21AI61379.

REFERENCES

1. Antinori, S., E. Gianelli, S. Calattini, E. Longhi, M. Gramiccia, and M. Corbellino. 2005. Cutaneous leishmaniasis: an increasing threat for travelers. Clin. Microbiol. Infect. 11:343–346.
2. Ballas, Z. K. 2007. Modulation of NK cell activity by CpG oligodeoxynucleotides. Immunol. Res. 39:15–21.
3. Belkaid, Y., C. A. Piccirillo, S. Mendez, E. M. Shevach, and D. L. Sacks. 2002. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 420:502–507.
4. Desjens, P. 2004. Leishmaniasis: current situation and new perspectives. Comp. Immunol. Microbiol. Infect. Dis. 27:305–318.
5. Ferlazzo, G., B. Morandi, A. D’Agostino, R. Meazza, G. Melioli, A. Moretta, and L. Moretta. 2003. The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of infected dendritic cells. Eur. J. Immunol. 33:306–313.
6. Gerosa, F., B. Baldoni-Guerra, C. Nisi, V. Marchesini, G. Carra, and G. Trinchieri. 2002. Reciprocal activating interaction between natural killer cells and dendritic cells. J. Exp. Med. 195:327–333.
7. Granucci, F., I. Zanoni, N. Pavelka, S. L. Van Dommelen, C. E. Andoniu, F. Belardelli, M. A. Degli Esposti, and P. Ricciardi-Castagnoli. 2004. A contribution of mouse dendritic cell-derived IL-2 for NK cell activation. J. Exp. Med. 200:287–295.
8. Granucci, F., I. Zanoni, and P. Ricciardi-Castagnoli. 2006. Natural killer (NK) cell functions can be strongly boosted by activated dendritic cells (DC). Eur. J. Immunol. 36:2819–2820.
9. Kallinaki, P., A. Giermasz, Y. Nakamura, P. Basse, W. J. Storkus, J. M. Kirkwood, and R. B. Mailliard. 2005. Helper role of NK cells during the induction of antitumor responses by dendritic cells. Mol. Immunol. 42:535–539.
10. Laskay, T., A. Diefenbach, M. Rollinghoff, and W. Sobh. 1995. Early parasite containment is effective for resistance to Leishmania major infection. Eur. J. Immunol. 25:2220–2227.
11. Latz, M. B., N. Kukutsch, A. L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow infection. J. Immunol. Methods 223:77–92.
12. Mattner, J., N. Donhauser, G. Werner-Felmayer, and C. Bogdan. 2006. NKT cells mediate organ-specific resistance against Leishmania major infection. Microbes Infect. 8:354–362.
13. Mendez, S., K. Tabbara, Y. Belkaid, S. Bertholet, D. Verthelyi, D. Klinnman, R. A. Seder, and D. L. Sacks. 2003. Coinjection with CpG-containing immunostimulatory oligodeoxynucleotides reduces the pathogenicity of a live vaccine against cutaneous leishmaniasis but maintains its potency and durability. Infect. Immun. 71:5121–5129.
14. Momeni, A. Z., and M. Aminjavaheri. 1995. Treatment of recurrent cutaneous leishmaniasis. Int. J. Dermatol. 34:129–133.
15. Moretta, L., G. Ferlazzo, M. C. Mingari, G. Melioli, and A. Moretta. 2003. Update on natural killer cells: cross-talk with dendritic cells and role in the cure of acute myeloid leukemias. Cancer J. 9:232–237.
16. Piccioli, D., S. Sbrana, E. Melandri, and N. M. Valiante. 2002. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. J. Exp. Med. 195:335–341.
17. Pillarsetty, V. G., S. C. Katz, J. I. Bleier, A. B. Shah, and R. P. Damatteo. 2005. Natural killer dendritic cells have both antigen presenting and lytic function and in response to CpG produce IFN-gamma via autocrine IL-12. J. Immunol. 174:2612–2618.
18. Ramezani-Pineda, J. R., A. Frohlich, C. Berberich, and H. Moll. 2004. Dendritic cells (DC) activated by CpG DNA ex vivo are potent inducers of host resistance to an intracellular pathogen that is independent of IL-12 derived from the immunizing DC. J. Immunol. 172:6281–6289.
19. Sacks, D., and N. Nohlen-Truth. 2002. The immunology of susceptibility and resistance to Leishmania major in mice. Nat. Rev. Immunol. 2:845–858.
20. Satoskar, A. R., L. M. Stamm, X. Zhang, A. A. Satoskar, M. Okano, C. Terhorst, J. R. David, and B. Wang. 1999. Mice lacking NK cells develop an inefficient Th1 response and control cutaneous Leishmania major infection. J. Immunol. 162:6747–6754.
21. 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.
22. Scharton-Kersten, T., and P. Scott. 1995. The role of the innate immune response in Th1 cell development following Leishmania major infection. J. Leukoc. Biol. 57:515–522.
23. Schleicher, U., J. Mattner, M. Blos, H. Schindler, M. Rollinghoff, M. Karaghisioff, M. Muller, G. Werner-Felmayer, and C. Bogdan. 2004. Control of Leishmania major in the absence of Tyk2 kinase. Eur. J. Immunol. 34:519–529.
24. Sivori, S., M. Falco, M. Della Chiesa, S. Carlomagno, M. Vitalle, L. Moretta, and A. Moretta. 2004. CpG and double-stranded RNA trigger human NK cells by Toll-like receptors: induction of cytokine release and cytotoxicity against tumors and dendritic cells. Proc. Natl. Acad. Sci. USA 101:10116–10121.
25. Spath, G. F., and S. M. Beverley. 2001. A lipophosphoglycan-independent method for isolation of infective Leishmania metacyclic promastigotes by density gradient centrifugation. Exp. Parasitol. 99:97–103.
26. Stanley, A. C., Y. Zhou, F. H. Amante, L. M. Randall, A. Haque, D. G. Pellicci, G. R. Hill, M. J. Smyth, D. I. Godfrey, and C. R. Engwerda. 2008. Activation of invariant NKT cells exacerbates experimental visceral leishmaniasis. PLoS Pathog. 4:e1000028.
27. Trinchieri, G. 2003. Interleukin-12 and the regulation of innate resistance to adoptive immunity. Nat. Rev. Immunol. 3:133–146.
28. Wei, S., I. Kryczek, and W. Zou. 2006. Regulatory T-cell compartmentalization and trafficking. Blood 108:426–431.
29. Wu, W., L. Weigand, Y. Belkaid, and S. Mendez. 2006. Immunomodulatory effects associated with a live vaccine against Leishmania major containing CpG oligodeoxynucleotides. Eur. J. Immunol. 36:3238–3247.
30. Zanoni, I., M. Foti, P. Ricciardi-Castagnoli, and F. Granucci. 2005. TLR-dependent activation stimuli associated with Th1 responses confer NK cell stimulatory capacity to mouse dendritic cells. J. Immunol. 175:286–292.