Uptake of *Leishmania major* by dendritic cells is mediated by Fcγ receptors and facilitates acquisition of protective immunity

Florian Woelbing,1 Susanna Lopez Kostka,1 Katharina Moelle,1 Yasmine Belkaid,3 Cord Sunderkoetter,5 Sjef Verbeek,6 Ari Waisman,2 Axel P. Nigg,1 Juergen Knop,1 Mark C. Udey,4 and Esther von Stebut1

Uptake of *Leishmania major* by dendritic cells (DCs) results in activation and interleukin (IL)-12 release. Infected DCs efficiently stimulate CD4+ and CD8+ T cells and vaccinate against leishmaniasis. In contrast, complement receptor 3–dependent phagocytosis of *L. major* by macrophages (MΦ) leads exclusively to MHC class II–restricted antigen presentation to primed, but not naive, T cells, and no IL–12 production. Herein, we demonstrate that uptake of *L. major* by DCs required parasite–reactive immunoglobulin (Ig)G and involved FcγRI and FcγRII. In vivo, DC infiltration of *L. major*–infected skin lesions coincided with the appearance of antibodies in sera. Skin of infected B cell–deficient mice and Fcγ−/− mice contained fewer parasite–infected DCs in vivo. Infected B cell–deficient mice as well as Fcγ−/− mice (all on the C57BL/6 background) showed similarly increased disease susceptibility as assessed by lesion volumes and parasite burdens. The B cell–deficient mice displayed impaired T cell priming and dramatically reduced IFN–γ production, and these deficits were normalized by infection with IgG-opsonized parasites. These data demonstrate that DC and MΦ use different receptors to recognize and ingest *L. major* with different outcomes, and indicate that B cell–derived, parasite-reactive IgG and DC FcγRI and FcγRII are essential for optimal development of protective immunity.

In cutaneous leishmaniasis affecting mice and man, control of infection is associated with Th1/Tc1-mediated, IFN–γ–dependent elimination of intracellular parasites (1, 2). After infection of mice with physiologic low dose inocula of *Leishmania major* parasites, the evolution of skin lesions occurs in three distinct phases (3). In the initial “silent” phase, resident macrophages (MΦ) phagocytose *L. major* promastigotes primarily via complement receptor 3 (CR3) (4, 5), which activates the infected cells and allows parasite amplification (as intracellular amastigotes) at sites of infection. In the second phase, development of clinically evident lesions occurs coincident with the influx of inflammatory cells, including neutrophils, MΦ, and eosinophils. Subsequently, immunity is initiated by infiltration of DCs as well as T and B cells, and lesions resolve (the third phase) (3).

Both MΦ and DCs, the major APCs in skin, clearly influence the development of cellular immune responses against *Leishmania*. Dermal MΦ capture organisms at sites of inoculation and, after establishment of protective immunity, they ultimately kill the parasites. However, MΦ do not actively participate in T cell priming. In all likelihood, DCs take up amastigotes of *L. major* present in lesional skin, become activated, and migrate to draining LN where they present *Leishmania* antigen to naive T cells (6, 7). There are striking differences in the ways that MΦ and DCs interact with *L. major* parasites in vitro. First, skin DCs preferentially take up *L. major* amastigotes, the obligate intracellular life form of the parasite, rather than promastigotes (transmitted by sand flies), whereas MΦ efficiently

---

**Correspondence**

Esther von Stebut: vonstebu@mail.uni-mainz.de

Abbreviations used: BMDC, bone marrow–derived DC; CFSE, carboxyl fluorescein succinimidyl ester; CR3, complement receptor 3; IS, immune response; MΦ, macrophages; NMS, normal mouse serum; SLA, soluble *Leishmania* antigen.

F. Woelbing and S.L. Kostka contributed equally to this paper.
phagocytose both life forms (7–9). Second, the phagocytic capacity of DCs is limited with regard to efficiency and capacity as compared with that of MΦ (7). Third, _L. major_-infected DCs, unlike infected MΦ, release IL-12 and efficiently induce Th1/Tc1 differentiation of naive cells (7, 10–12). Fourth, although both cell types present _Leishmania_ antigen via the MHC class II pathway, only DCs prime and restimulate _L. major_-specific CD8⁺ T cells (13).

Based on the different behaviors and functional roles of MΦ and DCs in _L. major_ infections, we hypothesized that DCs and MΦ might take up _L. major_ via different phagocytic receptors. MΦ ingest _L. major_ via CR3-dependent mechanisms (4). Herein, we identified immune IgG and Fcγ receptors (FcγRI and FcγRIII) as critical mediators of _L. major_ uptake by DCs in vitro. In vivo, mice infected with IgG-opsonized parasites showed enhanced protective immunity as well as increased numbers of _L. major_-infected lesional DCs. We also determined that B cell– (μMT and JHT) and Fcγ-deficient mice had decreased numbers of _L. major_-infected lesional DCs and enhanced lesion progression. In addition, we observed impaired CD4- and CD8-priming in the absence of B cells. Immune IgG production and engagement of DC FcγR are required for timely development of Th1/Tc1-dependent immunity and control of experimental cutaneous leishmaniasis in mice.

**RESULTS**

**CR3 does not mediate uptake of _L. major_ by DCs**

MΦ phagocytosis of _L. major_ promastigotes and amastigotes is rapid and efficient (1). In contrast, skin DCs preferentially ingest amastigotes, and this occurs slowly and inefficiently (7). We generated bone marrow–derived DCs (BMDCs) using GM-CSF/IL-4 and confirmed our previous findings obtained with skin DCs. Day 6 immature DCs expressed CD11c, intermediate levels of MHC class II, and low levels of CD86 (Fig. 1 A). BMDCs, like skin DCs, internalized freshly isolated amastigotes in a time- and dose-dependent manner. Normal mouse serum (NMS)–opsonized promasti-gotes, in contrast, were not readily ingested (27 ± 6 vs. 8 ± 1% infected DCs with a DC/parasite ratio of 1:3 at 18 h; _P_ ≤ 0.05, Fig. 1 B). As expected, DC infection was associated with up-regulation of MHC class I/II and costimulatory markers (reference 7 and unpublished data).

Phagocytosis of _L. major_ by MΦ is CR3 dependent (5). To investigate the role of CR3 and CR4 in _L. major_ uptake by DCs, we used CD18⁻/⁻ mice. As expected, DCs generated from CD18⁻/⁻ mice did not express CD11b or CD11c (unpublished data). No differences in the percentages of infected wild type or CD18⁻/⁻ DCs (Fig. 1 C) or the number of parasites/cell was observed after DCs and _L. major_ amastigotes were cocultured for 18 h.

We also assessed the involvement of other candidate receptors. Antibodies reactive with CD11b (clone M1/70) (9), CD205 (clone NLDC145) (14), or preincubation with mannan (5) were used at optimal concentrations. This concentration of mannan was able to completely inhibit the uptake of _C. albicans_ by MΦ (unpublished data) (5). None of the inhibitors tested affected the uptake of _L. major_ by DCs (Fig. 1 D). Thus, CR3/CR4 and C-type lectins appear to be dispensable for phagocytosis of _L. major_ by DCs.

**Immunoglobulins enhance uptake of _L. major_ by DCs**

_L. major_ amastigotes are isolated from infected tissues, whereas metacyclic promastigotes are enriched from stationary phase in vitro cultures. Among the most prominent differences between surface characteristics is the large amount of Ig bound

---

**Figure 1.** _L. major_ amastigotes, rather than promastigotes, are preferentially internalized by DCs independent from CR3/CR4. Bone marrow–derived DCs and amastigotes or promastigotes of _L. major_ were cocultured at various ratios at 2 × 10⁶ DCs/ml. (A) Before coculture, surface phenotypes of immature DCs were verified by FACS. (B) At the indicated time points, cells were harvested, cytospun, and the percentage of infected cells was determined (mean ± SEM, _n_ ≥ 3, *, _P_ ≤ 0.05, **, _P_ ≤ 0.005, ***, _P_ ≤ 0.002). (C) DCs from CD18⁻/⁻ and wild-type 129 × C57BL/6 controls were cocultured with _L. major_ (1:3). (D) C57BL/6 DC were preincubated with 5 mg/ml mannan, 50 μg/ml anti-CD11b, anti-DEC205, or isotype control before amastigotes of _L. major_ were added (1:3). [C and D] After 18 h, cells were harvested and cytospins were analyzed for the percentage of infected DC (mean ± SEM, _n_ ≥ 3, *, _P_ ≤ 0.05).
to the surfaces of amastigotes, but not promastigotes. To determine if Ig was involved in parasite uptake, we quantified the ability of amastigotes isolated from B cell-replete, wild-type BALB/c mice, μMT (B cell–deficient) mice and SCID (B cell– and T cell–deficient) mice to parasite DCs. DCs readily phagocytosed amastigotes from BALB/c mice, but not amastigotes from μMT or SCID mice (Fig. 2, A and B). Opsonization with NMS did not affect uptake. Parasites from B cell–deficient mice efficiently entered DCs only after they had been preincubated with Ig-containing immune serum (IS) from L. major–infected BALB/c mice (or C57BL/6 mice; unpublished data). Phagocytosis of amastigotes by MΦ was not affected by the presence or absence of Ig. Opsonization of amastigotes from B cell–deficient mice with IS (Fig. 2 C) also induced enhanced release of IL-12p40 from DCs, whereas infection of MΦ did not promote IL-12 production. Ig-mediated uptake of amastigotes did induce IL-10 release from MΦ (15), whereas little, if any, IL-10 was produced by infected DCs.

To determine if Ig-coated promastigotes could be ingested by DCs, metacyclic L. major promastigotes were left untreated or were opsonized with NMS or IS for 10 min at 37°C. After washing, parasites were cocultured with DCs for 18 h. IS-treated promastigotes were efficiently taken up by DCs and induced IL-12 release, whereas untreated or NMS-treated parasites were not ingested (Fig. 3 A). Interestingly, complete transformation of promastigotes into amastigotes was not observed within all DCs, even after 18 h (Fig. 3 B), suggesting that there are differences in the phagosomal compartments of DCs and MΦ that influence this transition (e.g., differences in pH, content of proteolytic enzymes) (16).

Stimulation of antigen-specific, carboxyl fluorescein succinimidyl ester (CFSE)-labeled T cells with parasite-treated DCs revealed that DCs infected with NMS amastigotes or IS promastigotes induced similar expansion of both CD4+ and CD8+ T cells, whereas DCs treated with NMS promastigotes did not promote T cell proliferation (Fig. 3 C).

Phagocytosis of L. major is IgG dependent
Amastigotes from infected tissue efficiently parasitize DCs. By flow cytometry, we detected both IgG1 and IgG2a/b on the surfaces of amastigotes when analyzed directly after isolation from C57BL/6 mice or after opsonization with NMS (Fig. 3 D). Additional opsonization with NMS or IS led to enhanced binding of IgM to amastigotes. Cell surface Ig was not detected on promastigotes. NMS-opsonized promastigotes exhibited surface-associated IgM, attributable to natural Ig found in the sera of naive mice (17). Because both NMS- and IS-opsonized amastigotes were taken up to similar extents and NMS-opsonized promastigotes were not phagocytosed by DCs (compare with Fig. 3 A), we conclude that IgM is not required for parasite uptake. Interestingly, promastigotes bound similar amounts of IgG1 and IgG2a/b when incubated with sera harvested from infected resistant C57BL/6 mice (Fig. 3 D) or susceptible BALB/c mice (unpublished data).

To conclusively implicate immune IgG in DC-parasite uptake, we isolated total IgG from IS using protein G affinity columns and tested the capacity of IgG to trigger phagocytosis. Similar to IS, the IgG fraction mediated uptake of promastigotes, whereas parasites incubated with the IgG-depleted fraction were not phagocytosed (Fig. 3 E). In addition, parasite uptake was associated with IL-12p40 release (772 ± 324 pg/ml for DCs incubated with IgG promastigotes vs. 137 ± 27 pg/ml for DCs cocultured with promastigotes incubated with the IgGneg fraction; n ≥ 3, P = 0.03).
Both FcγRI and FcγRIII mediate uptake of *L. major* by DCs

IgG1-containing immune complexes bind preferentially to FcγRIII (and FcγRII) and IgG2a-containing complexes bind with higher affinity to FcγRI than to FcγRIII. FcγRII typically mediates endocytosis of soluble immune complexes (18). DCs from knockout mice deficient for single FcγR family members ingested *L. major* as efficiently as DCs from wild-type mice (Fig. 4 A). In addition, blocking antibodies directed against FcγRI/III (clone 2.4G2) did not have a dramatic effect on *L. major* uptake by wild-type DCs (Fig. 4 B, left). However, significant inhibition of *L. major* phagocytosis by DCs (up to 70%) was observed if DCs from FcγRI/III- or Fcγ-deficient mice were compared with wild-type cells (Fig. 4 B). Uptake of amastigotes and Ig-opsonized promastigotes was impaired to similar extents. Thus, FcγRI and FcγRIII each facilitate phagocytosis of *L. major* by DCs, and these receptors can compensate for one another.

Accumulation of infected DCs in lesions coincides with the appearance of *Leishmania*-specific IgG in sera

In the setting of physiologic low dose infections, we have shown that increased accumulation of both T cells and DCs at inoculation sites coincides with the onset of lesion involution (3). In addition, infiltration with DCs was delayed as compared with MΦ recruitment and infection. DCs were identified in lesions beginning 5 wk after inoculation, and their number increased substantially during the healing phase.

To determine if immune IgG, which dramatically enhances *L. major* infection of DCs in vitro, is present at the time that DCs are recruited to *Leishmania* lesions, we infected C57BL/6 mice with *10^3* promastigotes and quantified the number of inflammatory cells in lesional skin as well as the appearance of *Leishmania*-reactive IgG in sera at weekly intervals. Fig. 5 shows that by weeks 5–6 after infection, the numbers of DCs as well as serum parasite-specific IgG levels were increased. This indicates that *Leishmania*-specific IgG is available to opsonize parasites and enhance phagocytosis by DCs at the time that DCs are infected in vivo. Significant accumulation of CD19^+ B cells in lesional skin (*>10^3* cells) was not detected within 8 wk after infection.

In vivo targeting of DCs with IgG-opsonized promastigotes speeds disease resolution

Previously, we and others have demonstrated that *L. major*-infected DCs release IL-12 and effectively vaccinate against...
progressive disease (10–12). Therefore, infection of DCs in vivo earlier in the course of infection should accelerate development of Th1 immunity. To test this hypothesis, promastigotes were opsonized either with NMS or IS. After washing, low dose infections using 10³ opsonized parasites were initiated in the ear skin of C57BL/6 mice (Fig. 6). Inflammatory dermal cells from lesional ear skin were studied weekly (Fig. 6, A and B). Interestingly, the numbers of CD11c⁺ DCs in IS promastigote–infected skin were significantly higher (7.9 ± 0.9 × 10⁵/lesion) than those in NMS promastigote–treated ears (2.5 ± 0.5 × 10⁵/lesion in week 1, n = 3, P ≤ 0.005), especially at early time points. At later time points (week 3 and after), this difference was not evident. DCs were enriched by preparative flow sorting and the number of infected DCs was determined (mean ± SEM, n = 3, *, P ≤ 0.05, **, P ≤ 0.005 compared with isotype treatment [B, left] or wild type control [B, right]).

Lesion development in infected mice was monitored for >3 mo. Interestingly, cutaneous lesions of mice infected with IS-opsonized parasites were significantly smaller and resolved more quickly than those in mice that were infected with parasites opsonized with NMS (Fig. 6 D). In addition, lesional parasite loads were decreased in weeks 4 and 6 after infection in mice inoculated with IS-opsonized parasites compared with NMS-treated parasites (Fig. 6 E). Smaller lesion volumes were associated with increased Th1 immunity as measured by antigen–specific restimulation of LN cells at weeks 4 and 6 (Fig. 6 F). The IFN-γ/IL-4 ratio in IgG-parasite infected mice was Th1-predominant (week 6: 1.068 ± 250) as compared with mice infected with NMS-opsonized parasites (week 6: 382 ± 86, n = 6, P ≤ 0.05). Collectively, these data suggest that enhanced IgG-mediated recruitment and L. major infection of DCs in vivo leads to enhanced Th1 immunity and more rapid resolution of cutaneous lesions.

B cell–deficient mice show enhanced lesion progression associated with decreased numbers of infected DCs and impaired CD4⁻ and CD8⁻-priming

Because our data suggested that IgG mediates parasite uptake by DCs, we characterized L. major infections in B cell–deficient μMT mice (19). Herein, wild-type C57BL/6 or μMT mice were infected with physiologically relevant doses of L. major (10⁴ promastigotes). Compared with wild-type mice, μMT mice showed significantly enhanced lesion progression from week 6 after infection (Fig. 7 A). Lesion involution was delayed by ~4 wk in μMT compared with control mice. Furthermore, the skin of μMT mice contained greater numbers of parasites reaching a peak load of 4 ± 2 × 10³ parasites/ear at week 6 as compared with 3 ± 2 × 10³ parasites/ear in wild types (P ≤ 0.05) (Fig. 7 B). The IFN-γ/IL-4 ratios of μMT LN cell cultures stimulated with soluble Leishmania antigen (SLA) were also skewed toward a Th2 profile as compared with C57BL/6 cells. In weeks 6 and 8 after infection, μMT LN cells released significantly less IFN-γ and more IL-4 compared with C57BL/6 mice (e.g., 40.1 ± 12.6 in μMT compared with 100.7 ± 19.2 ng IFN-γ/ml in C57BL/6 mice in week 6, n ≥ 9, P ≤ 0.05; Fig. 7 C).
Increased recruitment and infection of DCs after injection of IgG-opsonized promastigotes of *L. major* leads to enhanced protection in vivo. C57BL/6 mice were infected intradermally into ear skin with $10^5$ metacyclic promastigotes that have been opsonized with normal mouse serum (NMS) or serum from 6 wk–infected BALB/c mice (IS) and washed. Inflammatory ear cells were isolated and analyzed for the presence of CD11c$^+$ DCs at different time points by flow cytometry (A). The number of DCs per ear was calculated (mean ± SEM, n = 3, **, P ≤ 0.005; B). CD11c$^+$ DCs were purified using flow sorting and cytospun. The percentage of infected DCs was determined by light microscopy (mean ± SEM, n = 3, ***, P ≤ 0.002; B and C). (D) Lesion development was monitored over the course of >3 mo. Lesion volumes are shown as mean ± SEM (**, P ≤ 0.005 and ***, P ≤ 0.002, n ≥ 10 mice). (E) Parasite loads of infected mice were determined at the indicated time points using limiting dilution assays. Each data point represents the number of organisms from one ear and the bars indicate arithmetic means. One representative out of two independent experiments is shown. (F) Cytokine levels were determined in LN cultures stimulated in the presence of soluble Leishmania antigen by ELISA (mean ± SEM, *, P ≤ 0.05, n ≥ 10).

We also isolated inflammatory cells from infected ears of μMT and wild-type mice. No significant difference in the number of CD11c$^+$ DCs that accumulated in the lesions of μMT mice as compared with C57BL/6 ears was found (unpublished data). However, lesions of μMT mice contained significantly fewer *L. major*–infected DCs at several time points (Fig. 7 D). Finally, we sought to determine if there was a correlation between numbers of infected DCs and the ability to prime CD4 and CD8 T cells in situ. LN cells of infected C57BL/6 or μMT mice were isolated 6 wk after infection and labeled with CFSE. Antigen-specific expansion of CD4$^+$ and CD8$^+$ T cells was assessed 5 d after restimulation of LN cells with SLA. μMT LN cells exhibited decreased SLA-specific CD4$^+$ T cells as was also greatly reduced in the absence of B cells (SLA: 2.9 ± 0.5% compared with 14.1 ± 3.9% in C57BL/6 mice, n = 5, P ≤ 0.05). In summary, enhanced lesion progression in the μMT mice was associated with decreased numbers of infected DCs and defective T cell priming.

Infection of μMT mice with IgG-opsonized parasites normalizes lesion development

To investigate whether the deficiency in B cells or the lack of antibody contributed to the phenotype of μMT mice, we infected μMT mice with $10^4$ NMS- or IS-opsonized promastigotes. In this setting, μMT mice infected with *L. major* developed lesions in the presence of immune IgG that were significantly smaller than those caused by NMS-opsonized parasites (Fig. 8 A). In parallel, decreased lesion volumes in IgG-opsonized parasite-infected μMT mice correlated with significantly smaller parasite burdens in week 6 (Fig. 8 B). In IS parasite–infected μMT mice, the IFN$\gamma$/IL-4 ratio was shifted from a Th2-predominant (828 ± 94) to a Th1 immune response (3,680 ± 1,515, n = 4, week 6). Thus, the lack of host IgG is responsible for disease outcome in μMT mice. The skin of μMT mice infected with NMS-opsonized or IS-opsonized promastigotes was analyzed for the presence of infected CD11c$^+$ DC (Fig. 8 C). As shown before, infection of maximally 5% of DCs was found in μMT mice infected with NMS-treated parasites. Interestingly, inoculation of IgG–containing parasites led to dramatically increased numbers of infected DCs in the early course of infection (Fig. 8 C), even higher than those found in wild types (compare with Fig. 7 D).

The μMT mice were previously shown to contain Ig in the sera, at least when mice were of BALB/c genetic background (20, 21). The presence of soluble Ig is due to low-level leakiness of the locus (21). To confirm critical
In experiments in a truly B cell-deficient mouse strain, we
infected C57BL/6 JHT mice characterized by deletion of the
Ig heavy chain (22). As shown in Fig. 8 D, increased lesion
development was observed in JHT mice over the course of
4 wk identical to the course of infections in μMT mice. This
is in contrast with the findings of Miles et al., who reported
that JH mice on a BALB/c background were less susceptible
to infection than their controls (23).
Enhanced lesion progression and decreased numbers of infected DCs in vivo in mice lacking Fc receptors

Our data suggested that FcγR-mediated uptake of L. major parasites by DCs mediates protection. Thus, we infected Fcγ chain–deficient mice lacking all three known activating FcR with physiologically low dose inocula of L. major (Fig. 8 E). Lesions were monitored for >3 mo. FcγR−/− C57BL/6 mice developed more progressive lesions between weeks 4 and 9 as compared with wild-type controls. Maximum lesion sizes in FcγR−/− mice were detected in week 9, reaching 21 ± 2 mm³ (C57BL/6: 13 ± 1 mm³, n = 14, P ≤ 0.008). Increased lesion volumes were paralleled by significantly higher parasite burdens as determined in week 4 after infection (Fig. 8 E).

Similar to the course of disease in B cell–deficient mice, lesion involution in FcγR−/− mice was normal and all mice ultimately healed their infection. This data suggests that FcγR-mediated antibody effects are not an absolute requirement for healing.

Finally, we assessed the number of parasite-containing CD11c+ DC in lesions of FcγR−/− mice infected for 4 wk with low doses of L. major (Fig. 8 E). Ear skin of FcR–deficient mice harbored fewer parasite-infected DC (10.5 ± 2.3%) as compared with wild-type DCs (20.2 ± 3.8%, n = 4, P = 0.09). This finding confirmed our in vitro data obtained with BMDCs generated from FcγR−/− mice that demonstrated inhibited parasite uptake in cocultures with L. major (Fig. 4 B).

**DISCUSSION**

Microbe-binding receptors orchestrate events that occur subsequent to phagocytosis by transducing specific cellular signals (24). The main receptor for uptake of Leishmania promastigotes by MΦ is CR3 (4, 5). In the initial stages of cutaneous leishmaniasis, most parasites are taken up by MΦ. CR3-mediated phagocytosis of Leishmania by MΦ leads to selective inhibition of IL-12 release (5, 25–27). Production of IL-12 in infected and lysed MΦ by CR3 is presumed to be dependent on production of specific antibody as well.

In MΦ, ingestion of amastigotes, in contrast with CR3-phagocytosed promastigotes, appears to occur through both the FcγR and CR3 (15, 37). In our work and consistent with prior findings, IgG did not play an important role in the uptake of amastigotes from SCID versus BALB/c mice by inflammatory skin MΦ (38). Our results also confirm the finding that IgG-mediated phagocytosis of L. major by MΦ leads to strong release of IL-10, and no IL-12 synthesis (15), which might promote parasite survival (39). Thus, FcγR-mediated uptake by MΦ and DCs is opposing roles in initiating immune responses in cutaneous leishmaniasis.

The role of B cell–derived IgG in cutaneous leishmaniasis in vivo is not fully understood yet. Polyclonal activation of human B cells leads to the production of large amounts of parasite-specific and nonspecific Ab, particularly IgM and IgG (40). Also, amastigotes released into lesional tissue from infected and lysed MΦ appear to be coated with antiparasite antibodies (41). In this study, we show that Leishmania–specific IgG was present in sera at the time of DC accumulation in lesions. Consistent with prior findings, intradermal infection with IgG-opsonized parasites led to enhanced early recruitment of CD11c+ DCs into the lesions (38), most likely by IgG–triggered chemokine release from MΦ (42, 43). Administration of IgG-opsonized parasites also led to enhanced infection of DC, augmented T cell priming, and limited disease as compared with inoculation of IgG–free parasites.

Prior data and our experiments suggest that IgG-mediated effects differ significantly, dependent on the genetic background of the mice. B cell–deficient JH BALB/c mice showed improved disease outcome after infection with supraphysiologic doses of L. pijanoi and coinjection of anti–Leishmania IgG reversed their phenotype (44). Administration of IgG at or near the time of parasite inoculation worsened disease outcome in BALB/c mice (40, 45, 46). This is consistent with studies demonstrating that FcγR ligation on infected MΦ induced IL-10 release, which in turn prevented parasite elimination and promoted disease progression (15, 23).

We were unable to implicate mannan–binding C-type lectins in phagocytosis of L. major by murine DCs.

In this study, we demonstrate that L. major parasites are predominantly phagocytosed by DCs via FcγRI and FcγRIII. In line with several studies, FcγR ligation was associated with DC activation and IL-12 release (32–34). We have previously shown that DCs can cross-present Leishmania antigen to CD8+ T cells (13), whereas CR3-mediated phagocytosis by MΦ leads exclusively to MHC class II–restricted antigen presentation. These results bear some similarity to experiments evaluating the role of FcγR in antitumor immunity. In FcγR−/− mice, effective cross-presentation of tumor antigens by DCs was also dependent on FcγR–dependent activation (35). In addition, signaling through FcγRI/III facilitated efficient restimulation of tumor–reactive T cells (36). Thus, cross-presentation of both tumor-derived and L. major-associated antigens by DCs requires FcγR, and is presumably dependent on production of specific antibody as well.
proof was provided by the demonstration that anti-*Leishmania* IgG reconstitution of Jfl BALB/c mice correlated with increased IL-10 production and blocking of IL-10R prevented antibody-mediated disease exacerbation (23).

Mice on a *Leishmania*-resistant background lacking functional B cells (e.g., μMT C57BL/6 mice) did not exhibit a phenotype with regard to lesion development after high dose infection with *L. major* (19, 37, 47, 48). However, DeKrey et al. reported that C57BL/6 μMT mice infected with high-dose inocula of *L. major* showed reduced IFN-γ production after pathogen challenge (48). In our experiment, using physiologically relevant low dose inocula, μMT as well as Jfl T C57BL/6 mice consistently exhibited enhanced lesion progression and delayed lesion involution, higher parasite loads, and cytokine profiles consistent with a Th2-predominant response as compared with C57BL/6 mice. In accordance with our in vitro data, significantly fewer infected DCs were found in lesions of μMT mice. In addition, we determined that in the absence of *Leishmania* IgG-mediated infection of DCs, decreased numbers of *Leishmania*-reactive CD4+ and CD8+ T cells developed. The defects observed in μMT mice were reversed by using IgG-opsonized parasites for infection indicating that the deficiency in Ig is responsible for worsened disease outcome in B cell–deficient mice.

As expected from the in vitro results obtained with BM-DCs generated from Fcγ-deficient mice, we also found decreased numbers of infected DC in Fcγ−/− mice paralleled by increased lesion volumes over the course of several weeks and higher parasite burdens. In contrast, in prior studies, improved disease outcome of Fcγ−/− mice was observed using infections with *L. pifanoi* or *L. major* (44, 49). However, the mice used were on a BALB/c background and, thus, are not comparable to those used for this study. Data generated with *Leishmania*-resistant mice might be more physiologically relevant in a clinical setting because the course of disease in, for example, C57BL/6 mice more closely mimics *L. major* infections of humans.

In summary, we propose that the two predominant APCs in skin, MΦ and DCs, are sequentially engaged via different pathogen recognition receptors as cutaneous leishmaniasis evolves. Although in the initial “silent” phase, *L. major* promastigotes are primarily phagocytosed by resident MΦ via CR3, FcyR and DCs become critically important in established infections. IgG-mediated uptake of *L. major* by DCs leads to IL-12 production and priming of Th1/Tc1 cells, both of which are required for efficient parasite killing by le- sional MΦ. In contrast, FcyR-mediated uptake of amas- tigotes by MΦ induces counter-regulatory IL-10 production. This may facilitate activation of regulatory T cells, which, in turn, promotes parasite persistence and maintenance of T cell memory (39, 50). The balance between CR3 and FcyR- triggered anti- and proinflammatory mechanisms involving MΦ and DCs is critical for disease outcome. The unexpected identification of immune IgG production as a prerequisite for efficient cross-priming of *Leishmania*-specific Th1/Tc1 cells is intriguing. In future experiments it will be important to assess the T cell dependence of *Leishmania*-reactive antibody production, and to identify the APCs that are involved in B cell and, if relevant, Th priming.

**MATERIALS AND METHODS**

**Animals.** 6–8-wk-old BALB/c and C57BL/6 mice were purchased from the Central Animal Facility of the University of Mann. CD18−/− mice (51) on a mixed C57BL/6 and 129/SV background were provided by K. Scharf- fettner-Kochanek (Department of Dermatology, University of Ulm, Ulm, Germany). FcγRII−/− mice (52) were obtained from H. Mossmann (Max Planck-Institut für Immunobiologie, Freiburg, Germany). Mice deficient for FcyRIII (53) and FcyRI (54) as well as FcyRI/III double deficient mice (all C57BL/6 background) were provided by S. Verbeek. C57BL/6 Fcγ−/− were obtained from T. Saito (RIKEN Research Center for Allergy and Immunology, Yokohama, Japan) (55) or from Taconic. B cell–deficient mice (C57BL/6 SCID, μMT, JflT) were gifts from M. Neurath, K. Stenbrink, and A. Wasmann (all from University of Mann, Mann, Germany). All ani- mals were housed in accordance with institutional and federal guidelines. All experiments were undertaken with approved license from the Animal Care and Use Committee of the Region Rheinland-Pfalz.

**Cells.** Inflammatory skin-derived MΦ (MΦ) were elicited by subcutaneous injection of polyclonal antibodies and enriched to homogeneity (7). BMDCs were generated in GM-CSF– and IL–4–containing media (56) and harvested on day 6 of cell culture. The characteristics of the cell populations were assessed by flow cytometry using relevant surface markers. The following antibodies were used: anti-I-Ab/d/I-Ed (2G9), anti-CD11b (M1/70), anti-CD11c (HL3), anti-CD40 (3/23), anti-CD54 (3E2), anti-CD80 (1G10), anti-CD86 (GL1) (all from BD Biosciences/Becton Dickinson), anti-F4/80 (Serotec), and respective isotype control mAb.

**Parasites.** Metacyclic promastigotes or amastigotes of *L. major* clone VI (MHOM/IL/80/Friedlin) were prepared as described previously (25, 57). Amastigotes were prepared from infected footpad of BALB/c or C57BL/6 mice, or mice genetically deficient in B cells (μMT, SCID) to obtain para- sites devoid of Ig. Isolated parasites were opsonized with 5% NMS or serum from 6-wk-infected BALB/c or C57BL/6 mice (immune serum, IS) for 10 min (37°C) and washed before in vitro or in vivo infections. Parasites were stained for surface-associated Ig using isotype-specific secondary antibodies reactive with mouse Ig: anti-IgM (Serotec), anti-IgG1 (A85-1), and anti-IgG2a/b (B2-40, all from BD Biosciences). After staining, parasites were washed with PBS/2% BSA, fixed, and analyzed by flow cytometry. Anti- *Leishmania* IgG was prepared from pooled sera of ≥5–6-wk *L. major*–infected BALB/c mice using protein G columns (Pierce Chemical Co.) following the manufacturer’s protocol. Sera were stored at −20°C before IgG purification. Purified IgG was stored at 4°C (0.8 mg/ml) in PBS before use.

**Phagocytosis and inhibition studies.** Isolated cells were subcultured in medium (RPMI 1640/5% FCS) at 2 × 10⁵/ml and parasites were added at the parasite/cell ratios indicated. In some experiments, cells were preincu- bated for 60 min with mannan (Sigma-Aldrich, 1 and 5 mg/ml), anti-CD11b, anti-CD16/32, anti-CD205, or control rat IgG (all at 50 μg/ml, all from BD Biosciences). Cells were harvested after several hours and cytokines were measured. DiffQuick-stained cells were analyzed for the presence of intra- and extracellular parasites. At least 200 cells were counted per sample. Supernatants from parasite/cell cocultures were collected and assayed for the presence of IL-12p40 or IL-10 by ELISA (BD Biosciences).

**Assessment of B cell and DC infiltration and function in vivo.** Groups of 8–5 C57BL/6 mice were infected intradermally in ear skin with 1,000 *L. major* promastigotes. At several time points, ears were harvested and the number of B cells and DCs that had accumulated at the site of infection was determined (3). In brief, ears were incubated with 2 mg/ml Liberase (Boehringer Ingelheim). After 2 h, cells were dissociated mechanically and counted and the frequency of CD19+ and CD11c+ cells was assessed using
In vivo infections using IgG-opsonized parasites and B cell– or FcγRII-deficient mice. C57BL/6, μMT, Jj1T, or FcγRI/−/− mice were infected intra-

dermally with 10^6 metacyclic L. major promastigotes. In some experiments, parasites were opsonized for 10 min with either NMS or IS and washed. Lesion development was assessed weekly in three dimensions using a caliper, and lesional volumes were enumerated using limiting dilution assays (57). For measurement of cytokine production, 10^6 retro-
uentricular LN cells/200 μl were added to 96-well plates in the presence of SLA (25 μg/ml). Antigen-specific IFN-γ and IL-4 production was determined after 48 h using ELISA (R&D Systems).

At several time points, ears were harvested and inflammatory cells isolated using Liberase and mechanical disruption (3). The cells were counted and the frequencies of CD11c^+ DC were determined using flow cytometry. CD11c^+ cells were enriched to >98% purity using a high speed cell sorter (FACS Vantage SE System, Becton Dickinson) and cytospins were analyzed by light microscopy to estimate the number of infected DCs/ear.

The frequency of daughter cells of proliferating antigen-reactive compared with nonproliferating LN T cells was estimated using flow cytometry (S8–60). 6 wk after infection, LN cells were harvested and 5 × 10^5 cells/ml were labeled with 1 μM CFSE (Invitrogen). LN cells were subsequently plated at 10^5/200 μl media in a 96-well U-bottom plate and left untreated or stimulated with SEB (10 μg/ml; Sigma-Aldrich), or SLA (61). After 5 d, proliferation was determined using flow cytometry. T cells were selected for analysis using mAbs against CD4 (L3T4, RM4-5), CD8 (Ly2, 53–6.7), and CD11c. The frequency of daughter cells of proliferating antigen-reactive compared with nonproliferating cells was calculated.

Statistics. Statistical analysis was performed using the unpaired Student’s t test.

The authors wish to thank Dr. K. Reifenberg and staff for excellent help in the animal care facility, Dr. H.-J. Peter (Department of Dermatology, Charité Berlin) for providing technical assistance with IgG purification, Dr. D. Sacks for helpful discussions, and Drs. K. Steinbrink and H. Januleit for critically reading the manuscript.

This work was supported in part by grants from the Deutsche Forschungsgemeinschaft (DFG, SFB 490, and SFB 458) to E. von Stetub and by the Intramural Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research to M.C. Udey.

The authors have no conflicting financial interests.

Submitted: 15 November 2005
Accepted: 7 December 2005

REFERENCES

1. Reiner, S.L., and R.M. Lockley. 1995. The regulation of immunity to Leishmania major. Annu. Rev. Immunol. 13:151–177.

2. Sacks, D., and N. Noben-Trauth. 2002. The immunology of susceptibility and resistance to Leishmania major in mice. Nat. Rev. Immunol. 2:848–858.

3. Belkaid, Y., S. Mendez, R. Lira, N. Kadambi, G. Milon, and D.L. Sacks. 2000. A natural model of Leishmania major infection reveals a prolonged “silent” phase of parasite amplification in the skin before the onset of lesion formation and immunity. J. Immunol. 165:969–977.

4. Mosser, D.M., and P.J. Edelson. 1985. The mouse macrophage receptor for C3bi (CR3) is a major mechanism in the phagocytosis of Leishmania promastigotes. J. Immunol. 135:2785–2789.

5. Schonlau, F., K. Scharffetter-Kochanek, S. Grábbe, B. Prietz, C. Sorg, and C. Sunderkötter. 2000. In experimental leishmaniasis deficiency of CD18 results in parasite dissemination associated with altered macrophage functions and incomplete Th1 cell response. Eur. J. Immunol. 30:2729–2740.

6. Roll, H. 1993. Epidermal Langerhans cells are critical for immunoregulation of cutaneous leishmaniasis. Immunol. Today. 14:383–387.

7. von Stetub, E., Y. Belkaid, T. Jakob, D.L. Sacks, and M.C. Udey. 1998. Uptake of Leishmania major amastigotes results in activation and IL-12 release from murine skin-derived dendritic cells: implications for the initiation of anti-Leishmania immunity. J. Exp. Med. 188:1547–1552.

8. Locksley, R.M., F.P. Heinzel, J.E. Fankhauser, C.S. Nelson, and M.D. Sadick. 1988. Cutaneous host defense in leishmaniasis: interaction of isolated dendritic macrophages and epidermal Langerhans cells with the insect-stage promastigote. Infect. Immun. 56:336–342.

9. Blank, C., H. Fuchs, K. Rapporferger, M. Röllinghoff, and H. Roll. 1993. Parasitism of epidermal Langerhans cells in experimental cutaneous leishmaniasis with Leishmania major. J. Infect. Dis. 167:418–425.

10. Föbe, S.B., C. Bauer, S. Föbe, and H. Roll. 1998. Antigen-pulsed epidermal Langerhans cells protect susceptible mice from infection with the intracellular parasite Leishmania major. Eur. J. Immunol. 28:3800–3811.

11. Aluha, S.S., R.L. Reddick, N. Sato, E. Montalbo, V. Kosteczki, W. Zhao, M.J. Dolan, P.C. Melby, and S.K. Aluha. 1999. Dendritic cell (DC)-based anti-inflammatory strategies: DCs engineered to secrete IL-12 are a potent vaccine in a murine model of an intracellular infection. J. Immunol. 163:3890–3897.

12. von Stetub, E., Y. Belkaid, B.V. Nguyen, M. Cushing, D.L. Sacks, and M.C. Udey. 2000. Leishmania major-infected murine Langerhans cell-like dendritic cells from susceptible mice release IL-12 after infection and vaccinate against experimental cutaneous leishmaniasis. Eur. J. Immunol. 30:3498–3506.

13. Belkaid, Y., E. von Stetub, S. Mendez, R. Lira, E. Caler, S. Bertholet, M.C. Udey, and D.L. Sacks. 2002. CD8^+ T cells are required for primary immunity in C57BL/6 mice following low-dose, intradermal challenge with Leishmania major. J. Immunol. 168:3992–4000.

14. Bozza, S., R. Guazzano, A. Spreca, A. Bacci, C. Montagnoli, P. di Francesco, and L. Romani. 2002. Dendritic cell transport conduits and hypoxia of Apergillus fumigatus from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. J. Immunol. 168:1362–1371.

15. Kane, M.M., and D.M. Mosser. 2001. The role of IL-10 in promoting disease progression in leishmaniasis. J. Immunol. 166:1141–1147.

16. Desjardins, M., and G. Griffiths. 2003. Phagocytosis: latex leads the way. Curr. Opin. Cell Biol. 15:498–503.

17. Dominguez, M., and A. Torano. 1999. Immune adherence-mediated opsonophagocytosis: the mechanism of Leishmania infection. J. Exp. Med. 189:25–35.

18. Verbeck, J.S., W.L. Hazenbos, P.J. Capel, and J.G. van de Winkel. 1997. The role of FcR in immunity: lessons from gene targeting in mice. Int. Immunol. 14:466–474.

19. Brown, D.R., and S.L. Reiner. 1999. Polarized helper-T-cell responses against Leishmania major in the absence of B cells. Infect. Immun. 67:266–270.

20. Macpherson, A.J., A. Lamare, K. McCoy, G.R. Harriman, B. Odermatt, G. Dougan, H. Hengartner, and R.M. Zinkernagel. 2001. IgA production without μ or δ chain expression in developing B cells. Nat. Immunol. 2:625–631.

21. Hasun, M., B. Polic, M. Bralic, S. Jonjic, and K. Rajewsky. 2002. Incomplete block of B cell development and immunoglobulin production in mice carrying the μMT mutation on the BALB/c background. Eur. J. Immunol. 32:3463–3471.

22. Gu, H., Y.R. Zou, and K. Rajewsky. 1993. Independent control of immunoglobulin switch recombination at individual switch regions
evidenced through Cre-loxP-mediated gene targeting. Cell. 73:1185–1194.

23. Miles, S.A., S.M. Conrad, R.G. Alves, S.M. Jeronimo, and D.M. Mosser. 2005. A role for IgG immune complexes during infection with the intracellular pathogen Leishmania. J. Exp. Med. 201:747–754.

24. Mosser, D.M. 1994. Receptors on phagocytic cells involved in microbial recognition. Immunol. Rev. 60:99–114.

25. Belkaid, Y., B. Butcher, and D.L. Sacks. 1998. Analysis of cytokine production by inflammatory mouse macrophages at the single-cell level: selective impairment of IL-12 induction in Leishmania-infected cells. Eur. J. Immunol. 28:1389–1400.

26. Carrera, L., R.T. Gazzinelli, R. Badolato, S. Hieny, W. Muller, R. Kuhn, and D.L. Sacks. 1996. Leishmania promastigotes selectively inhibit IL-12 induction in bone marrow–derived macrophages from susceptible and resistant mice. J. Exp. Med. 183:515–526.

27. Sartori, A., M.A. Oliveira, P. Scott, and G. Trinchieri. 1997. Metacyclogenesis modulates the ability of Leishmania promastigotes to induce IL-12 production in human mononuclear cells. J. Immunol. 159:2849–2857.

28. Carrera, L., R.T. Gazzinelli, R. Badolato, S. Hieny, W. Muller, R. Kuhn, and D.L. Sacks. 1996. Leishmania promastigotes selectively inhibit IL-12 induction in bone marrow–derived macrophages from susceptible and resistant mice. J. Exp. Med. 183:515–526.

29. Sartori, A., M.A. Oliveira, P. Scott, and G. Trinchieri. 1997. Metacyclogenesis modulates the ability of Leishmania promastigotes to induce IL-12 production in human mononuclear cells. J. Immunol. 159:2849–2857.

30. Appelmelk, B.J., I. van Die, S.J. van Vliet, C.M. Vandenbroucke-SARTORI, A., M.A. Oliveira, P. Scott, and G. Trinchieri. 1997. Metacyclogenesis modulates the ability of Leishmania promastigotes to induce IL-12 production in human mononuclear cells. J. Immunol. 159:2849–2857.

31. Cambi, A., K. Gijzen, J.M. de Vries, R. Torensum, B. Joosten, G.J. Adema, M.G. Netea, B.J. Kullberg, L. Romani, and C.G. Figdor. 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells. Eur. J. Immunol. 33:532–538.

32. Romani, L., C. Montagnoli, S. Bozza, K. Perruccio, A. Spreca, P. Allavena, S. Verbeek, R.A. Calderone, F. Bistoni, and P. Puccetti. 2004. The exploitation of distinct recognition receptors in dendritic cells determines the full range of host immune relationships with Candida albicans. Int. J. Immunol. 16:149–161.

33. Colmeneraes, A.L., Corbi, S.J. Turco, and L. Rivas. 2004. The dendritic cell receptor DC-SIGN discriminates among species and life cycle forms of Leishmania. J. Immunol. 172:1186–1190.

34. Regnault, A., D. Lankar, V. Lacobanne, A. Rodriguez, C. Thery, M. Rescigno, T. Sato, S. Verbeek, C. Bonnerot, P. Kriciardi-Castagnoli, and S. Amigorena. 1999. Fcy receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I–restricted antigen presentation after immune complex internalization. J. Exp. Med. 190:371–380.

35. Sedlik, C., D. Orbach, P. Veron, E. Schweighoffer, F. Colucci, R. Gambirale, A. Ioan-Facsinay, S. Verbeek, P. Kriciardi-Castagnoli, C. Sunderkotter, and M.C. Udey. 2003. Interleukin 10 secretion and major histocompatibility complex class I–restricted immunity. J. Immunol. 170:1653–1639.

36. Cambi, A., K. Gijzen, J.M. de Vries, R. Torensum, B. Joosten, G.J. Adema, M.G. Netea, B.J. Kullberg, L. Romani, and C.G. Figdor. 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells. Eur. J. Immunol. 33:532–538.

37. Romani, L., C. Montagnoli, S. Bozza, K. Perruccio, A. Spreca, P. Allavena, S. Verbeek, R.A. Calderone, F. Bistoni, and P. Puccetti. 2004. The exploitation of distinct recognition receptors in dendritic cells determines the full range of host immune relationships with Candida albicans. Int. J. Immunol. 16:149–161.

38. Colmeneraes, A.L., Corbi, S.J. Turco, and L. Rivas. 2004. The dendritic cell receptor DC-SIGN discriminates among species and life cycle forms of Leishmania. J. Immunol. 172:1186–1190.

39. Regnault, A., D. Lankar, V. Lacobanne, A. Rodriguez, C. Thery, M. Rescigno, T. Sato, S. Verbeek, C. Bonnerot, P. Kriciardi-Castagnoli, and S. Amigorena. 1999. Fcy receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I–restricted antigen presentation after immune complex internalization. J. Exp. Med. 190:371–380.

40. Belkaid, Y., K.F. Hoffmann, S. Mendez, S. Kamhawi, M.C. Udey, T.A. Wynn, and D.L. Sacks. 2001. The role of interleukin (IL)-10 in the persistence of Leishmania major in the skin after healing and the therapeutic potential of anti-IL-10 receptor antibody for sterile cure. J. Exp. Med. 194:1497–1506.
α promotes Th1 differentiation and inhibits disease progression in Leishmania major-susceptible BALB/c mice. J. Exp. Med. 198: 191–199.

58. Chen, J.C., M.L. Chang, and M.O. Muench. 2003. A kinetic study of the murine mixed lymphocyte reaction by 5,6-carboxyfluorescein diacetate succinimidyl ester labeling. J. Immunol. Methods. 279:123–133.

59. Adkins, B., T. Williamson, P. Guevara, and Y. Bu. 2003. Murine neonatal lymphocytes show rapid early cell cycle entry and cell division. J. Immunol. 170:4548–4556.

60. Ritter, U., A. Meissner, C. Scheidig, and H. Korner. 2004. CD8 α-and Langerin-negative dendritic cells, but not Langerhans cells, act as principal antigen-presenting cells in leishmaniasis. Eur. J. Immunol. 34:1542–1550.

61. Mendez, S., S. Gurunathan, S. Kamhawi, Y. Belkaid, M.A. Moga, Y.A. Skeiky, A. Campos-Neto, S. Reed, R.A. Seder, and D.L. Sacks. 2001. The potency and durability of DNA- and protein-based vaccines against Leishmania major evaluated using low dose, intradermal challenge. J. Immunol. 166:5122–5128.