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Loss of Dendritic Cell Migration and Impaired Resistance to *Leishmania donovani* Infection in Mice Deficient in CCL19 and CCL21

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The encounter between APC and T cells is crucial for initiating immune responses to infectious microorganisms. In the spleen, interaction between dendritic cells (DC) and T cells occurs in the periarteriolar lymphoid sheath (PALS) into which DC and T cells migrate from the marginal zone (MZ) along chemokine gradients. However, the importance of DC migration from the MZ into the PALS for immune responses and host resistance to microbial infection has not yet been elucidated. In this study, we report that following *Leishmania donovani* infection of mice, the migration of splenic DC is regulated by the CCR7 ligands CCL19/CCL21. DC in *plt/plt* mutant mice that lack these chemokines are less activated and produce less IL-12, compared with those in wild-type mice. Similar findings are seen when mice are treated with pertussis toxin, which blocks chemokine signaling in vivo. *plt/plt* mice had increased susceptibility to *L. donovani* infection compared with wild-type mice, as determined by spleen and liver parasite burden. Analysis of splenic cytokine profiles at day 14 postinfection demonstrated that IFN-γ and IL-4 mRNA accumulation was comparable in wild-type and *plt/plt* mice. In contrast, accumulation of mRNA for IL-10 was elevated in *plt/plt* mice. In addition, *plt/plt* mice mounted a delayed hepatic granulomatous response and fewer effector T cells migrated into the liver. Taken together, we conclude that DC migration from the MZ to the PALS is necessary for full activation of DC and the optimal induction of protective immunity against *L. donovani*. The Journal of Immunology, 2006, 176: 5486–5493.

*Abbreviations used in this paper: DC, dendritic cell; HPRT, hypoxanthine-guanine phosphoribosyltransferase; LCMV, lymphocytic choriomeningitis virus; MZ, marginal zone; MZM, MZ macrophages; PALS, periarteriolar lymphoid sheath; p.i., postinfection; PTX, pertussis toxin; STAg, soluble *Toxoplasma gondii* Ag.*
conditions. B6 mice were purchased from Charles River Laboratories and bred at the London School of Hygiene and Tropical Medicine under barrier provided by H. Hengartner and T. Junt (University of Zurich, Switzerland) and amastigotes were isolated from infected hamsters, as previously described.

Flow cytometry
Spleens were harvested and digested in RPMI 1640 (Invitrogen Life Technologies) containing 0.05% collagenase (Worthington Biochemical) and 100 μg/ml DNase I (Sigma-Aldrich) at 37°C for 30 min. After washing with calcium-free medium, cells were stained for FITC-, PE-labeled, or biotinylated CD11c (HL3), I-A<sup>+</sup> (M5/117 and 20G9), CD40 (3/23), CD80 (16-10A1), and CD86 (GL1) (BD Pharmingen), followed by incubation with allophycocyanin-labeled streptavidin. Cells were analyzed using a FACSCalibur (BD Biosciences).

Immunohistochemistry
Immunohistochemistry was performed on 6-mm frozen sections, as described before (19). Primary Abs were purified or biotinylated HLA3 (mouse CD11c), 53-6.7 (CD8a), RM4-5 (CD4), Gr-1 (RB6-8C5) (BD Pharmingen), Cl:A3-1 (anti-F4/80), FA-11 (CD68), 3D6.112 (anti-CD169) (Sero- tec), C17.8 (anti-IL-12p40), ER-TR9 (anti-specific ICAM-3 grabbing non-integrin-related 1; a gift from G. Kraal, Free University, Amsterdam, The Netherlands), or hamster antisera to L. donovani amastigotes. Secondary Abs were biotinylated rabbit anti-rat IgG (Vector Laboratories), 10% mouse serum-containing goat anti-hamster IgG (for L. donovani; Vector Laboratories), biotinylated rabbit anti-rat IgG (for confocal microscope; DakoCytomation), Alexa 488-conjugated goat anti-hamster IgG, or Alexa 546-conjugated streptavidin (Molecular Probes). As appropriate, sections were developed with Vector Elite-ABC kit, followed by Vector 3,3'-diaminobenzidine substrate kit (Vector Laboratories), or directly viewed using a Zeiss Axiophot (510 confocal microscope). In some experiments, mice were injected i.v. with 200 μl of 5% (v/v) in 0.9% NaCl india ink (Rowney) to allow visualization of MZ macrophages in the spleen.

Restimulation assay
Spleens from mice infected for 7 days were digested with collagenase, as described above. Dead cells and RBC were removed using a Histopaque density gradient. A total of 2 × 10<sup>6</sup> spleen cells was incubated with or without 2 × 10<sup>6</sup> paraformaldehyde-fixed L. donovani amastigotes in 10% FCS (Sigma-Aldrich) containing RPMI 1640 medium in a 24-well plate at 37°C for 2 h. For proliferation assays, the cells were pulsed with 1 μCi of [H]thymidine for final 6 h of culture and then harvested onto glass fiber filters. The incorporated [H]thymidine was determined by liquid scintillation spectrometry (20). Supernatants from the assays were collected after a 72-h incubation. IFN-γ-specific ELISA was performed using IFN-γ ELISA kit (R&D Systems), as described previously (20).

RNA was isolated from spleen tissue using an RNeasy Mini Kit with on-column DNase digestion (Qiagen), according to the manufacturer’s instructions. RNA was reverse transcribed into cDNA, as described previously (22). Oligonucleotides (5’-3’) used for specific amplification were: IL-4, CCTCACGACACACGAAACA (sense) and TGGACTCTATC ATGGTGCA (antisense); IL-10, AGGGTTACTTGGGTGTGCA (sense) and CACAGGGGAAACCTGTGA (antisense); IL-12p40, GGAACACGACACGACAAT (sense) and AACTGTTGGGAGAAG TACGGATTG (antisense); IFN-γ, CTTCTGCGGCGCCTAGCTC (sense) and TAAAGCCGAGAAACCGCATG (antisense); and for amplification of the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (HPRT) were GTTGAGTACAGGCAGACTTGTG (sense) and GAT TCAACCTTGCCGCTATCTTAGGC (antisense). The number of cytokines and HPRT cDNA molecules in each sample was calculated by real-time RT-PCR using QuantiTect SYBR green master mix (Qiagen) and an ABI prism 7000 (Applied Biosystems), according to the manufacturers’ instructions. Standard curves were constructed with known amounts of cytokines and HPRT cDNA, and the number of cytokine molecules per 1000 HPRT molecules in each sample was calculated.

Results

L. donovani infection in plt/plt mice
First, we determined the outcome of infection with L. donovani in plt/plt mice compared with wild-type B6 mice. Fig. 1A shows the outcome of infection in the liver, an organ associated with acquisition of cell-mediated immunity to L. donovani and thus with the capacity to clear intracellular amastigotes (23). Compared with B6 mice, liver parasite burdens were significantly higher in plt/plt

Evaluation of granulomatous response
Paraffin sections of liver tissue, stained with H&E, were prepared by conventional methods. In some cases, frozen sections of liver tissue were stained with hamster anti-L. donovani serum. The granuloma response in the infected livers was graded as follows: 1) an infected Kupffer cell without cellular infiltrate; 2) an immature granuloma composing an infected Kupffer cell surrounded by a few inflammatory cells, but without organization; 3) a mature granuloma having an organized structure; or 4) an empty granuloma, in which amastigotes had been killed as a result of effective antileishmanial immunity (21). At least 25–50 high magnification fields per mouse were counted and evaluated.

Real-time RT-PCR
FIGURE 1. The course of L. donovani infection in B6 and plt/plt mice. B6 (●) and plt/plt (○) mice were injected with 2 × 10<sup>7</sup> L. donovani amastigotes, and parasite burden was measured in the liver (A) or the spleen (B) at the time points indicated. The data are expressed as mean ± SEM for four to eight mice pooled from two experiments. *p < 0.05.
mice at day 28 postinfection (p.i.), and although these mice eventually were able to resolve hepatic infection, even at day 56, amastigote numbers in the tissue remained somewhat higher than seen in B6 mice. In contrast to the curing response observed in the liver, the spleen is a site of parasite persistence, extending over the 56-day time period studied. Strikingly, although parasite numbers were equivalent in these two mouse strains at days 14 and 28, plt/plt mice ultimately failed to exert control over parasite growth, and at day 56 spleen parasite burden was ~5-fold higher in plt/plt mice than in B6 mice (Fig. 1B). These data indicate that plt/plt mice are more susceptible to L. donovani infection than B6 mice.

**Uptake of L. donovani amastigotes in the spleen of plt/plt mice**

In the spleen, L. donovani amastigotes were phagocytosed mainly by macrophages in the MZ (13). However, we have demonstrated previously that plt/plt mice are deficient in MZ macrophages (MZM), a highly phagocytic subset of macrophages in the MZ (24). To determine whether the lack of MZM influenced initial splenic infection in plt/plt mice, we determined the number and location of amastigotes in the spleen at 1 h postinfection, when blood clearance is essentially complete (13). Double immunohistochemistry to identify amastigotes within subsets of splenic macrophages illustrated that CD68⁺ macrophages in the MZ, but not F4/80⁺ red pulp macrophages, are mainly responsible for uptake of L. donovani in plt/plt mice (Fig. 2A and data not shown). The localization (Fig. 2B, left panel) and absolute number (Fig. 2B, right panel) of amastigotes in the spleen of plt/plt mice were not altered compared with wild-type mice, with uptake being predominantly within the MZ in both strains. Thus, the deficiency of MZM in plt/plt mice does not compromise initial splenic infection, and most amastigotes are taken up by alternate CD68⁺ macrophages in the MZ.

**Early IL-12p40 responses and migration of DC are impaired in plt/plt mice**

The pattern of infection observed in plt/plt mice was remarkably similar to that previously described in studies in which IL-12p40 was targeted by neutralizing Abs (14) or in IL-12p40-deficient mice (15). As IL-12p40 is produced solely by DC in the early phase of immunity to L. donovani (13), we examined whether IL-12p40 responses were intact in plt/plt mice, using immunohistochemistry to identify both the number and localization of cells producing this cytokine. IL-12p40⁺ cells were rarely observed in naive mice of either strain. Five hours after infection of B6 mice, IL-12p40⁺ cells were readily observed in the spleen, mainly localizing, as previously described (13), to the deep periarteriol

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**FIGURE 2.** Distribution of parasite-infected macrophages, IL-12p40 production, and DC following L. donovani infection of B6 and plt/plt mice. The distribution of L. donovani amastigotes (green; arrowheads) and CD68⁺ macrophages (red) in the spleens of B6 and plt/plt mice (A) at 1 h p.i. Original magnification is ×800. B, The distribution and number of parasites in B6 (■) or plt/plt (□) mice were determined from multiple spleen sections of individual mice. Data are expressed as mean ± SEM for three to five mice per strain. The distribution of IL-12p40-producing cells in the spleens of B6 (C) and plt/plt (D) mice at 5 h p.i. IL-12p40 (arrowhead) was visualized with immunohistochemistry (brown), and MZ macrophages were labeled with india ink (black). Original magnification is ×200. E, The number of IL-12p40-producing cells in B6 (□) or plt/plt (■) mice was determined. Data are expressed as mean ± SEM for three to five mice per strain. *, p < 0.05. F, IL-12p40 mRNA was detected by real-time RT-PCR in the spleen of naive and 5-h infected B6 (■) or plt/plt (□) mice. The values are expressed with mean ± SEM for three to five mice. *, p < 0.05. G–J, DC were stained using CD11c in naive B6 (G) and plt/plt (H) and in B6 (I) and plt/plt (J) mice at 5 h p.i. Dotted lines indicated the border of the MZ. Original magnification is ×200. wp, White pulp; rp, red pulp. Data are representative of one of three experiments.
region (Fig. 2C). In contrast, few IL-12p40+ cells were identified in the spleens of plt/plt mice, and those that were seen were localized in the MZ (Fig. 2D). To quantify this observation in more detail, we scored the number of IL-12p40+ cells in multiple tissue sections from multiple mice. As shown in Fig. 2E, the frequency of IL-12p40+ cells per white pulp section was significantly reduced in plt/plt mice compared with B6 mice. As an independent means of evaluating the IL-12p40 response, we used real-time RT-PCR to determine the accumulation of IL-12p40 mRNA in spleen samples from naive and infected B6 and plt/plt mice. This analysis confirmed at the level of mRNA expression that the IL-12p40 response of plt/plt mice was indeed significantly reduced (Fig. 2F).

To examine whether altered localization of IL-12-producing cells in plt/plt mice is associated with abnormal distribution of DC, we visualized DC in the spleen of B6 and plt/plt mice, with or without L. donovani infection. DC in noninfected B6 mice distributed mostly in the MZ, with a few found in the PALS, as previously reported (25) (Fig. 2G). In contrast, DC in plt/plt mice were absent from the PALS and accumulated in the MZ and red pulp (9) (Fig. 2H). After 5 h of infection, most DC in B6 mice had migrated into the PALS (Fig. 2I), but the distribution of DC in plt/plt mice was not altered following infection (Fig. 2J). These data demonstrate that DC in plt/plt mice fail to migrate into the PALS and rather accumulate in the MZ.

**Impaired IL-12 production in PTX-treated mice**

To further define the importance of chemokine-dependent migration of DC in the introduction of IL-12 production, we treated B6 mice with PTX, which blocks chemokine signaling (26). DC in PTX-treated mice failed to migrate from the MZ into the PALS after 5 h of L. donovani infection, and showed a scattered distribution throughout the white pulp and red pulp (data not shown). As shown in Fig. 3, IL-12 p40 mRNA accumulation did not increase in the spleen of infected B6 mice treated with PTX as compared with control infected mice. These findings further support the notion that chemokine signaling at the early stage of L. donovani infection is critical for DC migration and optimal IL-12 production from splenic DC.

**DC activation is impaired in plt/plt mice**

IL-12p40 production is only one of a number of alterations that accompany the activation and maturation of splenic DC. To extend this analysis, we evaluated the expression of CD80, CD86, and CD40 on splenic CD11chighMHC-IIhigh DC isolated from naive and infected B6 or plt/plt mice. As shown in Fig. 4, DC activation during early L. donovani infection of B6 and plt/plt mice. A, Spleen DC from B6 (left panels) and plt/plt (right panels) were identified as CD11c+ MHC class IIhigh and then analyzed for expression of CD80 (top), CD86 (middle), and CD40 (bottom). Histograms show representative staining of naive (dotted line) and 5-h infected (solid line) mice. Data are representative of three mice from three independent experiments. B, Mean fluorescence intensities (MFI) of MHC-II, CD80, CD86, and CD40 on spleen DC from infected B6 (■) and plt/plt (□) mice. Data are shown as fold increase over DC in naive mice. The values are expressed with mean ± SEM for three independent experiments. *, p < 0.05.

CD11chighMHC-IIhigh DC isolated from B6 mice 5 h p.i. have slightly, but reproducibly increased levels of expression of CD80 and CD40 compared with naive B6 mice. In contrast, CD86 shows a more significant response in these mice. DC in plt/plt mice were comparable in their expression of CD11c and MHC-II with those in B6 mice (data not shown, Fig. 4B). However, following infection, no alteration in expression of CD80 and CD40 was observed on DC from plt/plt mice. Furthermore, although CD86 was upregulated, the increase was relatively weak compared with that observed in B6 mice (Fig. 4B). Thus, by various criteria, DC in plt/plt mice appear to be muted in their response to L. donovani infection compared with those in B6 mice.

**Immune deviation in plt/plt mice**

To evaluate whether the restricted activation of DC observed in plt/plt mice was translated into defective T cell priming (27), we...
isolated spleen cells from naive and infected B6 and plt/plt mice and restimulated them with L. donovani Ags in vitro. We observed that both the proliferative response (Fig. 5A) and the Ag-dependent production of IFN-γ (Fig. 5B) were comparable in these two mouse strains. To extend this analysis to other cytokines and to determine without any in vitro bias whether any form of immune deviation had occurred in these mice, we used real-time RT-PCR to evaluate the accumulation of mRNA for IFN-γ, IL-4, and IL-10 in naive and infected B6 and plt/plt mice. At day 14 p.i., the level of IFN-γ mRNA accumulation was identical in both strains (Fig. 5C), mirroring the data obtained from in vitro cultured spleen cells. IL-4 has also been shown to be coexpressed at the early stages of L. donovani infection (28), and although IL-4 mRNA was detected in infected mice, no difference was observed between B6 and plt/plt mice (Fig. 5D). In contrast, when we measured IL-10 mRNA accumulation, it was evident that expression of this cytokine was significantly enhanced in plt/plt mice compared with B6 mice (Fig. 5E). Thus, plt/plt mice show immune deviation toward production of a cytokine with known ability to inhibit antileishmanial immunity (29).

Delayed hepatic granuloma formation in plt/plt mice

Expression of immunity to L. donovani is most evident in the liver, and studies with asplenic mice indicate that T cell priming and differentiation to effector T cells in the spleen contribute significantly to the hepatic immune response (C. Engwerda, A. Stanley, C. Alexander, and P. Kaye, unpublished observations). We therefore wished to determine whether potential changes to T cell priming in the spleen of plt/plt mice were translated into reduced hepatic immunity as expressed by granuloma function. Tissue sections from the livers of infected B6 and plt/plt mice were evaluated throughout the time course of infection, and granuloma maturation was quantitated. As shown in Fig. 6, granuloma maturation was significantly retarded in plt/plt mice compared with B6 mice. Although amastigotes were abundant in the liver of both strains of mice at day 14 p.i. (Figs. 1 and 6, A and D), in plt/plt mice there was minimal initiation of a granulomatous response around infected Kupffer cells (Fig. 6D). At day 28 p.i., granuloma formation was detected in both B6 and plt/plt mice (Fig. 6, B and E), and by day 56, empty granulomas were observed in B6 mice, but less frequently in plt/plt mice (Fig. 6, C and F). Although absolute number of inflammatory foci was similar (Fig. 6G), the delay in maturation was observed throughout the time course studied, and at day 56 p.i., almost 20% of infected foci in plt/plt mice had still failed to generate a significant histologic response (Fig. 6H). Immunohistochemistry indicated that the defect in granuloma formation was associated with a lack of infiltration of both CD4+ and CD8+ T cells, whereas the accumulation of F4/80+ macrophages was comparable (Fig. 7). These data suggest therefore that although IFN-γ production is equivalent in B6 and plt/plt mice, this response fails to efficiently drive granuloma maturation in plt/plt mice, possibly as a consequence of the elevated IL-10 response.

Discussion

DC are APC that localize at peripheral tissues throughout the body and migrate into the T cell areas of secondary lymphoid organs for presentation of Ag to T cells. Therefore, migration of DC is thought to be one of the most important events for the induction of protective immunity to pathogens. In the spleen, most blood-borne pathogens are

FIGURE 5. Cytokine responses in L. donovani-infected B6 and plt/plt mice. A, Spleen cells from naive and day 7 infected B6 and plt/plt mice were cultured for 72 h in the absence (□) or presence (△) of L. donovani amastigote Ag. Proliferation was determined by scintillation counting. B, IFN-γ was determined in culture dependents from restructured B6 and plt/plt mice in the absence (□) or presence (△) of L. donovani amastigote Ag. C–E, mRNA was extracted from the spleens of naive or day 14 p.i. B6 (dark hatch) or plt/plt (light hatch) mice, and accumulation of IFN-γ (C), IL-4 (D), and IL-10 (E) mRNA was detected by real-time RT-PCR. *, p < 0.05.
trapped within the MZ, where macrophages and DC are abundantly distributed (30). DC in the MZ, activated following i.v. infection of LPS or soluble *Toxoplasma* Ag (STAg), rapidly move to the T cell area and form tight clusters with T cells (31, 32). These movements of DC are tightly controlled by chemokines and the regulation of chemokine receptor expression. For example, DC migration after LPS administration was not seen in *plt/plt* mice (9), and injection of STAg into CCR5-deficient mice failed to recruit DC into the PALS (33). Other animal studies also showed that the distribution of DC in the spleen was altered when they were infected with malaria (34), *Toxoplasma* (35), *Salmonella* (36), or lymphocytic choriomeningitis virus (LCMV) (37). However, none of these studies with live infectious agents have defined which chemokines are responsible for the observed migration of DC, how altered migration affects the functional behavior of these DC, or the consequences for host protection.

**FIGURE 6.** Granuloma formation in the *L. donovani*-infected livers of B6 and *plt/plt* mice. Liver sections at day 14 p.i. (A and D), day 28 p.i. (B and E), and day 56 p.i. (C and F), in B6 (A–C) or *plt/plt* (D–F) mice were stained with anti-*L. donovani* sera. Original magnification is ×400. G, The number of hepatic granulomas in infected B6 (■) or *plt/plt* (□) mice at each time point. H, Granuloma maturation during *L. donovani* infection of B6 or *plt/plt* mice. Data represent the frequency of infected Kupffer cells (iKC), immature granulomas (IG), mature granulomas (MG), and empty granulomas (EG) per mouse at each time point.

**FIGURE 7.** Hepatic T cell and macrophage recruitment during *L. donovani* infection of B6 and *plt/plt* mice. Liver sections from B6 (A–C) or *plt/plt* (D–F) mice at day 14 p.i. were stained for CD4 (A and D), CD8α (B and E), and F4/80 (C and F). Original magnification is ×200. Data are representative of one of three experiments.
In a mouse model of visceral leishmaniasis, we and other groups have demonstrated previously that macrophages in the MZ of the spleen phagocytosed the majority of parasites in the first hours after infection (13, 38), whereas IL-12-producing DC were observed deep in the T cell area of the spleen (13). These findings strongly suggested that either a small proportion of infected DC, or DC that had captured parasite-derived Ags in the MZ, migrated to the PALS, where they could encounter T cells for priming host-protective immune responses. In this study, we have demonstrated that the chemokines CCL19/21 are necessary for the migration of DC in this early phase of L. donovani infection. Many aspects of DC migration and activation in Leishmania infection appear different from that induced by LPS or STAg, however. In contrast to LPS or STAg administration, L. donovani infection induces migration of only a fraction of the DC population, and clustering of DC in the PALS is not as evident as with these other stimuli. Whereas DC in wild-type mice up-regulated both MHC class II and costimulatory molecules homogeneously at 5 h p.i., DC from plt/plt mice can up-regulate MHC class II to a similar extent, but a notably poor response was observed in terms of CD86 expression (Fig. 2). Another study of L. donovani infection using MyD88-deficient mice, which lack this common signaling pathway of TLR, has indicated that activation of DC was severely impaired, but that migration from the MZ to the PALS was not affected by loss of TLR signaling (39). These facts suggest there are at least two different stimuli acting on DC in L. donovani infection, which differentially affect DC migration and activation phenotype.

In plt/plt mice, although IL-12-producing cells were seen after 5 h of infection, the total amount of IL-12p40 mRNA was markedly decreased compared with that induced in B6 mice. In addition, all IL-12p40-producing DC were localized at the MZ in plt/plt mice. These findings indicate that partially activated DC can produce only small amounts of IL-12p40, whereas DC that have migrated into the PALS may become more fully activated and produce large amounts of IL-12p40. Although not studied in this work, this result may also imply that IL-23, which uses the IL-12p40 receptor, has indicated that activation of DC was severely impaired, but that migration from the MZ to the PALS was not affected by loss of TLR signaling (39). These facts suggest there are at least two different stimuli acting on DC in L. donovani infection, which differentially affect DC migration and activation phenotype.

Given the above findings, it was also surprising to observe that the induction of IFN-γ and IL-4, key cytokines involved in optimal host resistance and granuloma development following L. donovani infection (16, 45), was similar in both wild-type and plt/plt mice. Thus, the defects reported in this study in plt/plt mice clearly do not block T cell priming completely, as anticipated from other studies (46). In contrast, the accumulation of mRNA for IL-10, a cytokine with notable inhibitory effects on host resistance (28, 47), was increased in plt/plt compared with wild-type mice. IL-10 has multiple cellular sources during active infection with L. donovani, with IL-10 mRNA being most abundant (on a cell per cell basis) in CD4+ T cells, NK cells, DC, and macrophages, and to a lesser extent in CD8+ T cells, B cells, and neutrophils (A. Maroo, M. Svensson, S. Stager, and P. Kaye, unpublished observations). However, the broad expression of IL-10, difficulties associated with identifying IL-10-producing cells directly ex vivo, and a similarly wide cellular distribution of IL-10R expression together pose significant challenges for identifying functionally relevant cellular interactions mediated through IL-10. These are only likely to be addressable in vivo by the future development of mice, allowing cell-specific and regulated targeting of IL-10 and its receptor.

Resolution of hepatic infection is dependent on granuloma formation, in which various effector cells are recruited and produce cytokines. Both CD4+ and CD8+ T cells are required to induce granuloma formation in the liver (21). plt/plt mice have delayed granulomatous responses and an associated reduction in the recruitment of effector T cells. The lack of T cell recruitment is unlikely to be due to a lack of CCL19/21 in the liver, because CCL21 is detected in afferent lymphatics in the liver of plt/plt mice to the same extent as in the livers of wild mice (data not shown) (11). Rather, our data suggest that this lack of granuloma maturation is directly linked either to the altered functional development of effector CD4+ and/or CD8+ T cells, or their homing potential in an IL-10-rich environment. These speculations may be supported by the fact that IL-10 suppresses granuloma formation and recruitment of effector cells to the infected liver, independently of IFN-γ production (47).

In conclusion, plt/plt mice have a deficiency in DC activation resulting from impaired CCL21/CCL19-dependent migration of DC in these mice, which, most likely acting in concert with established defects in T cell migration (9), leads to a demonstrable increase in susceptibility to L. donovani infection. This study,
therefore, reveals for the first time the potential link between migration-dependent DC activation and protection against L. donovani infection, substantiating the importance of an appropriate chemokine environment for the generation and expression of optimal host-protective immune responses.

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Disclosures
The authors have no financial conflict of interest.

References