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Impairment of Alternative Macrophage Activation Delays Cutaneous Leishmaniasis in Nonhealing BALB/c Mice

Christoph Hölscher,*† Berenice Arendse,* Anita Schwegmann,* Elmarie Myburgh,* and Frank Brombacher2*†

Expressed on various cell types, the IL-4Rα is a component of both receptors for IL-4 and IL-13. Susceptibility of BALB/c mice to Leishmania major is believed to be dependent on the development of IL-4- and IL-13-producing Th2 cells, while IFN-γ secretion by Th1 cells is related to resistance. Despite a sustained development of Th2 cells, IL-4Rα-deficient BALB/c mice are able to control acute leishmaniasis, suggesting that IL-4Rα-bearing cells other than Th2 cells contribute to susceptibility. To analyze the contribution of the IL-4Rα on macrophages, recently generated macrophage/neutrophil-specific IL-4Rα-deficient mice on a susceptible BALB/c genetic background were infected with L. major. Strikingly, macrophage/neutrophil-specific IL-4Rα-deficient mice showed a significantly delayed disease progression with normal Th2 and type 2 Ab responses but improved macrophage leishmanicidal effector functions and reduced arginase activity. Together, these results suggest that alternative macrophage activation contributes to susceptibility in cutaneous leishmaniasis. The Journal of Immunology, 2006, 176: 1115–1121.

Leishmania major is the organism that has been most intensely studied and from which the original Th1/Th2 paradigm of immunological development during infectious disease was determined (1). Protective immunity against L. major is dependent on an IL-12-driven cell-mediated immune response mainly characterized by the production of IFN-γ in CD4+ Th1 cells and type 1 Ab isotypes in B cells. IFN-γ in turn mediates protection by inducing NO synthase-2 (NOS-2) expression and NO production in classically activated macrophages. In contrast, an IL-4-driven Th2 response and associated cytokines such as IL-13 counterregulate Th1 responses, and consequently, it would be expected that a Th2 response would be detrimental to the outcome of disease. Because susceptible BALB/c mice deficient for IL-4 (2), IL-13 (3), the IL-4Rα (4), or STAT6 (5) are able to contain infection with L. major downstream, IL-4Rα-mediated mechanisms became the center of interest. The IL-4Rα is a common component of the receptor complexes for IL-4 and IL-13. Accordingly, IL-4 and IL-13 have many functional properties in common, including the modulation of Th2 cell development, type 2 IgG class switching in B cells, and inflammatory responses due to the regulation of macrophage functions (6). However, although IL-4Rα−/− BALB/c mice control acute leishmaniasis, these mice still develop a Th2 response following infection (7). Thus, IL-4Rα-mediated mechanisms other than inducing Th2 responses must account for disease progression in L. major-infected BALB/c mice. One likely IL-4Rα-mediated mechanism could be the suppression of classical macrophage activation (caMtb) after L. major infection.

To distinguish the role of IL-4Rα signaling in specific cellular populations in vivo, we have generated macrophage/neutrophil-specific IL-4Rα−/− (LysMCreIL-4Rαlox/lox) mice to be in a position to differentiate IL-4Rα-dependent functions on the cellular level in vivo (8). Macrophages can be activated by different stimuli, with IFN-γ leading to caMtb and IL-4/IL-13 resulting in alternative macrophage activation (aaMtb) (reviewed in Ref. 9). Accordingly, we have recently demonstrated that LysMCreIL-4Rαlox/lox mice showed impaired aaMtb, which is essential for down-modulation of cell-mediated immune responses, immunopathology, and survival during schistosomiasis (8).

In this study, LysMCreIL-4Rαlox/lox mice on a susceptible BALB/c genetic background were infected with L. major to explore a possible role of aaMtb in cutaneous leishmaniasis. In contrast to susceptible BALB/c mice, LysMCreIL-4Rαlox/lox BALB/c mice showed a significantly delayed disease progression after infection with L. major concomitant with normal Th2 and type 2 Ab immune responses but improved macrophage leishmanicidial activities. These results suggest that alternatively activated macrophages are contributing to the susceptible phenotype in nonhealer BALB/c mice.

Materials and Methods

Mice
All experimental animals were bred under specific-pathogen-free conditions at the University of Cape Town (Cape Town, South Africa). IL-4Rα−/− (4) and conditional IL-4Rαlox/lox (8) mice were on a pure BALB/c genetic background. To obtain cell-specific IL-4Rα-deficient BALB/c mice, LysMCrelox/lox (10) on an F9 BALB/c genetic background were intercrossed with conditional IL-4Rαlox/lox BALB/c mice and further mated with complete IL-4Rα−/− BALB/c mice to generate hemizygous LysMCreIL-4Rαlox/− BALB/c mice. LysMCre-negative IL-4Rαlox/lox BALB/c mice (referred to as IL-4Rαlox/− BALB/c mice) and C57BL/6 mice were used as wild-type controls. For infection experiments, mice

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3 Abbreviations used in this paper: NOS-2, NO synthase-2; caMtb, classical macrophage activation; aaMtb, alternative macrophage activation; pLN, popliteal lymph node; BMMtb, bone marrow-derived macrophage; p.i., postinfection; RNI, reactive nitrogen intermediate.

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were matched for age and sex and maintained under barrier conditions in the biosafety level 2 facility in individually ventilated cages.

**Infection with L. major and histopathology**

*L. major* LV 39 (MHR0/Sv/vs9/P-strain) was maintained and prepared for infection as described previously (4). Anesthetized mice were infected s.c. into one hind footpad with 2 × 10⁶ stationary phase metacytic *L. major* promastigotes. Swelling of infected footpads was measured weekly using a Mitutoyo micrometer caliper. To calculate the parasite burden in the draining popliteal lymph nodes (pLN) from individual mice at different time points, pLN cells were resuspended in 6.4 ml of medium. One hundred microtrolers of 24, 2-fold serial dilutions were cultured at 26–28°C in microtiter plates containing 50 μl of a solid layer of rabbit blood agar. After 10 days, each individual well was scored microscopically for parasitic growth, and the fraction of negative wells per eight wells was determined for each dilution and subjected to statistical analysis for the calculation of minimal estimates of the number of viable *L. major* per lymph node by applying Poisson statistics and the χ² minimization method as described (11). For histopathological analysis, the infected footpad and a sample of liver were taken at different time points after infection and processed as described elsewhere (4).

**Determination of Ab isotypes**

Blood was collected and serum was prepared using serum separator tubes (BD Biosciences). Ag-specific IgG1, IgG2a, and IgG2b were quantified by ELISA, as previously described (7). Detection limits were 5 ng/ml for IgG1 and IgG2b and 0.1 ng/ml for IgG2a and IgG3. Total IgG was determined as described (4). The detection limits were 5 ng/ml for IgG1 and IgG2b, 0.1 ng/ml for IgG2a and IgG3, and 8 ng/ml for IgE.

**Restimulation assays**

Single-cell suspensions from draining pLNs were isolated by straining through a 40-μm cell strainer (BD Biosciences) and cultured in triplets at 2 × 10^5/ml in 48-well plates. Restimulation was performed with *L. major* Ag (10^5/ml inactivated parasites) or LPS (Sigma-Aldrich; 15 ng/ml). After 48 h, the content of IFN-γ, IL-4, and NO was quantified in culture supernatants (8). Arginase activity was determined by incubating 25 μl of 0.5 ML-arginine (Merck; pH 9.7) at 37°C for 60 min. The reaction was stopped with 400 μl of 0.1% Triton X-100 (Sigma-Aldrich). After 30 min on ice, the supernatant was removed, and the absorbance was measured at 450 nm. L-arginine hydrolysis was conducted by incubating 25 μl of 0.5 ml l-arginine (Merck; pH 9.7) at 37°C for 60 min. The reaction was stopped with 400 μl of H₂SO₄ (96%)/H₃PO₄ (85%)/H₂O (1/10/90), followed by heating at 95°C for 45 min. One unit of arginase activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol urea/min.

**Infection of bone marrow-derived macrophages (BMMΦ)**

BMMΦ were generated and triplicate cultures were infected with *L. major* promastigotes in a parasite-to-cell ratio of 10:1 as published (13). After 24 h, extracellular parasites were removed and cells were incubated for 16 h with medium or IL-4 (BD Biosciences; 1000 U/ml). Macrophages were subsequently stimulated with IFN-γ (BD Biosciences; 100 U/ml) and LPS (15 ng/ml). After 48 h, supernatants were collected for quantification of NO, and the amount of parasites was determined as described (13).

**Statistics**

Data are expressed as means of individual determinations and SDs. Statistical analysis was performed using unpaired Student’s *t* test defining differences to IL-4Rα/−/− mice as significant (*p* ≤ 0.05; **p** ≤ 0.01; ***p** ≤ 0.001).

**Results**

To understand IL-4Rα-mediated mechanisms in distinct cell types leading to suppression of protective immune responses during cutaneous leishmaniasis, macrophage/neutrophil-specific IL-4Rα-deficient (Ly5.1<sup>−/−</sup>Il-4Rα<sup>−/−</sup>) BALB/c mice were infected with 2 × 10<sup>6</sup> *L. major* metacytic promastigotes (Fig. 1). Healing C57BL/6 mice initially showed a transient and moderate footpad swelling but generated resistance to *L. major* infection (Fig. 1a) with low amounts of parasites in draining pLN (Fig. 1b). Susceptible nonhealing IL-4Rα<sup>−/−</sup> BALB/c mice developed progressive footpad swelling (Fig. 1a) and showed an enhanced parasite load in draining pLN (Fig. 1b) accompanied by ulceration and necrosis requiring termination of the experiment 11 wk after infection (Fig. 1c). In contrast, IL-4Rα/−/− BALB/c mice controlled acute *L. major* infection as previously shown (4) with moderate footpad swelling (Fig. 1a) and significantly reduced parasite burden in draining pLN (Fig. 1b) in the absence of ulceration and necrosis (Fig. 1c). Significantly, LysM<sup>−/−</sup>IL-4Rα<sup>−/−</sup> BALB/c mice showed an increased resistance, compared with susceptible IL-4Rα<sup>−/−</sup> BALB/c mice and (BALB/c mice, data not shown) with a similar moderate footpad swelling as observed in IL-4Rα<sup>−/−</sup> BALB/c mice for up to 13 wk after infection (Fig. 1a). Importantly, the parasite load in draining pLN from LysM<sup>−/−</sup>IL-4Rα<sup>−/−</sup> BALB/c control mice (Fig. 1b). Similar results were found in the footpad at week 6 postinfection (p.i.; data not shown). Thereafter, LysM<sup>−/−</sup>IL-4Rα<sup>−/−</sup> BALB/c mice developed disease progression with increasing footpad swelling accompanied by ulceration and necrosis (Fig. 1c) as well as elevated parasite burden in draining pLN (Fig. 1b) requiring termination of the experiment only at week 18. Histopathological analysis at this late time point demonstrated a similar inflammatory response in infected tissue accompanied with necrosis, bone destruction and inflammatory foci around disseminated pathogens in other organs as observed in IL-4Rα<sup>−/−</sup> BALB/c mice 9 wk earlier (Fig. 1c). These data reveal that IL-4Rα-mediated mechanisms in macrophages are involved in the development of early disease progression after *L. major* infection.

As Th2 and type 2 Ab immune responses were originally implicated with the fatal outcome of cutaneous leishmaniasis (2), we addressed whether delayed disease progression in LysM<sup>−/−</sup>IL-4Rα<sup>−/−</sup> BALB/c mice was accompanied by an altered Th1/Th2 and type1/type2 Ab immune response. Therefore, we analyzed humoral (Fig. 2) and cellular (Fig. 3) immune responses after infection with *L. major*. As determined by ELISA, 9 wk after infection with *L. major*, Leishmania-specific Ab isotypes detected in sera of infected LysM<sup>−/−</sup>IL-4Rα<sup>−/−</sup> BALB/c mice were similar to those observed in susceptible control IL-4Rα<sup>−/−</sup> BALB/c mice (Fig. 2), showing dominant type 2 Ab responses (IgG1 and IgE). In contrast, IL-4Rα<sup>−/−</sup> BALB/c mice developed a dominant type 1 isotype response (IgG2a, IgG2b). After Ag-specific restimulation, lymphocytes from IL-4Rα<sup>−/−</sup>IL-4Rα<sup>−/−</sup> and IL-4Rα<sup>−/−</sup> LysM<sup>−/−</sup>IL-4Rα<sup>−/−</sup> BALB/c mice expressed comparable amounts of IL-4 (Fig. 3). LysM<sup>−/−</sup>IL-4Rα<sup>−/−</sup> BALB/c mice, however, produced a marked IFN-γ response, which was also observed in healing C57BL/6 mice but not in susceptible IL-4Rα<sup>−/−</sup> or IL-4Rα<sup>−/−</sup> BALB/c mice (Fig. 3). This may suggest that proinflammatory macrophages may promote Th1 responses mediating protection in experimental cutaneous leishmaniasis.

caMΦ plays an important role in combating infection with *L. major* through IFN-γ-induced expression of effector molecules such as NOS-2-dependent NO and other reactive nitrogen intermediates (RNI) (14–16). This induction is counterregulated by IL-4Rα-dependent mechanisms, leading to caMΦ and the induction of arginase I. Because IL-4Rα-induced arginase I hydrolyzes l-arginine to urea and l-ornithine, this IL-4Rα-mediated pathway
IL-4R

ferences to IL-4R

/H9251

ter infection C57BL/6, IL-4R

anisms, we conducted a leishmanicidal assay in BMM

ulated that, in the absence of IL-4R

arginine as the substrate for NOS-2 (17, 18). Therefore, we spec-

ness in macrophages.

rophages from C57BL/6, IL-4R

creIL-4R

infected LysMcreIL-4R

jor

the production of NO was increased in macrophages from

BMM (Fig. 5). After in vitro infection with L. major promastigotes,

were preincubated with medium or IL-4 and subsequently

ated mechanisms appear to directly suppress leishmanicidal effec-

on macrophages, LysMcreIL-4R

duced susceptibility in nonhealer BALB/c mice. Due to the absence of IL-

4R-dependent competition with arginase I, our results suggest a

duced aaMφ in IL-4Rα mutant mouse strains. Vice versa, macro-

phages from C57BL/6, IL-4Rα−/− and LysMcreIL-4Rαbox−/− BALB/c mice produced significantly increased amounts of NO in

response to LPS, compared with susceptible IL-4Rαbox+/− BALB/c mice (Fig. 4b), suggesting that classical macrophage effector

mechanisms were enhanced in the absence of IL-4Rα responsiveness

In accordance with biased caMφ in the absence of the IL-4Rα

on macrophages, LysMcreIL-4Rαbox−/− BALB/c mice should be

able to better control L. major infection. Together, IL-4Rα-mediated

mechanisms appear to directly suppress leishmanicidal effector

functions in macrophages and may therefore contribute to the

enhanced susceptibility in nonhealer BALB/c mice. To evaluate

this IL-4Rα-dependent suppression of macrophage effector mecha-

isms, we conducted a leishmanicidal assay in BMMφ from IL-

4Rαbox−/−, IL-4Rα−/−, and LysMcreIL-4Rαbox−/− BALB/c mice (Fig. 5). After in vitro infection with L. major promastigotes,

BMMφ were preincubated with medium or IL-4 and subsequently

ulated with IFN-γ/LPS. Whereas BMMφ from all groups were

able to eliminate intracellular Leishmania in response to IFN-γ/LPS,

parasite elimination was strikingly inhibited by IL-4 in IL-

4Rαbox−/− BMMφ. In contrast, in vitro IFN-γ/LPS-mediated leish-

manicidal activity was unaffected by the addition of IL-4 in infected IL-4Rα−/− and LysMcreIL-4Rαbox−/− BMMφ (Fig. 5).

Moreover, IL-4Rα-mediated suppression of leishmanicidal effector

functions in BMMφ from IL-4Rαbox−/− mice correlated with a

decreased production of IFN-γ/LPS-induced NO. In contrast, NO

production by IFN-γ/LPS-stimulated and L. major-infected IL-

4Rα−/− and LysMcreIL-4Rαbox−/− macrophages could not be

hibited by IL-4 (Fig. 5). Together, our data provide conclusive

evidence that alternatively activated macrophages were not able to

ress a leishmanicidal effector and may therefore accelerate the

development of cutaneous leishmaniasis in nonhealer BALB/c mice.

Discussion

The differentiation of naive Th cells to cytokine-expressing effec-
tor cells is important for the orchestration of immunity. Th1 and

Th2 cells, which express the signatory (or archetypal) cytokines

IFN-γ and IL-4, respectively, mediate the release of type 1 or type

FIGURE 1. L. major infection. Experimental mice

were s.c. infected into one hind footpad with L. major. a,

Footpad swelling was calculated as the difference be-

tween the infected and the uninfected footpad. Data re-
present means and SDs of eight mice per group. b, After

9 and 18 wk p.i., the parasite burden in draining pLN

was determined by 2-fold limiting dilutions of single-
cell suspensions. c, At the indicated time points (b), mice were killed and samples from infected footpads

and liver were processed for histopathology (insets, parasites; arrows, inflammatory foci). One experiment representative of three performed is shown. Statistical analysis was performed using unpaired Student’s t test defining differences to IL-4Rαbox−/− (*, p ≤ 0.05; ***, p ≤ 0.001) or IL-4Rα−/− (†, p ≤ 0.05) BALB/c mice as significant.
FIGURE 2. Ab response after infection with L. major. Experimental mice were s.c. infected with L. major, and specific IgGs and total IgE Abs were quantified 9 wk p.i. Data represent means and SDs of eight mice per group. One experiment representative of three performed is shown.

2 Ab isotypes by B cells and promote the defense against distinct types of antigenic challenges, with Th1 cells more typically involved in systemic, and Th2 cells involved in mucosal immunity. Murine L. major infection remains the primary model for investigation of Th subset development because resistance and susceptibility have been definitively linked to Th cell differentiation; resistant healing mice generate a protective Th1 response, whereas susceptible mice develop a nonprotective Th2 response (19). Nevertheless, despite the usefulness of this model, the paradigm of Th1-mediated protection vs Th2-dependent susceptibility remains elusive because a number of alternative mechanisms to account for nonhealing disease have been postulated. Particularly, studies using neutralizing anti-IL-4 Abs or IL-4 neutralizing Abs showed an increased resistance, compared with susceptible IL-4-deficient (LysMcreIL-4Rα−/−) BALB/c mice as significant (***, p ≤ 0.001; ***, p ≤ 0.001).

FIGURE 3. Th cytokine response in L. major-infected mice. Experimental mice were s.c. infected with L. major, and specific cytokines were determined 9 wk p.i. The production of IFN-γ and IL-4 was determined after 48 h. Data represent means and SDs of triplicate cultures. One experiment representative of two performed is shown. Statistical analysis was performed using unpaired Student’s t test defining differences to IL-4Rα+ BALB/c mice as significant (**, p ≤ 0.01; ***, p ≤ 0.001).

IL-4−/− BALB/c mice has also been a controversial subject. Whereas Noben-Trauth et al. (21) have shown that IL-4−/− BALB/c mice develop progressive lesions and could not contain parasites, Kopf et al. (2) reported that IL-4−/− mice are able to resist infection. The use of parasite strains of different virulence may have partially accounted for this contrasting outcome of infection in IL-4−/− mice (22). Importantly, studies in IL-4Rα−/− BALB/c mice which are, despite a profound Th2 and type 2 Ab response, resistant to L. major infection, suggest that IL-4Rα-bearing cells other than lymphocytes are involved in IL-4Rα-dependent susceptibility (7). Moreover, comparative infection studies using IL-4−/− and IL-4Rα−/− or IL-13−/− and IL-4/IL-13−/− mice demonstrated that IL-13 contributes to disease progression in BALB/c mice during L. major (3, 4) and Leishmania mexicana infection (23). As murine lymphocytes are not responsive to IL-13, other cell types would appear to be involved in these functions. To understand IL-4Rα-mediated mechanisms in distinct cell types leading to suppression of protective immune responses during cutaneous leishmaniasis, macrophage/neutrophil-specific IL-4Rα-deficient (LysMcreIL-4Rα−/−) BALB/c mice were infected with L. major. Significantly, LysMcreIL-4Rα−/− BALB/c mice showed an increased resistance, compared with susceptible IL-4Rα−/− BALB/c mice for up to 13 wk after infection. However,
although disease progression was significantly delayed in L-lysinerich IL-4Rα-deficient BALB/c mice, macrophage/neutrophil-specific IL-4Rα-deficient BALB/c mice became eventually susceptible, whereas complete IL-4Rα-deficient BALB/c mice remained resistant to infection with L. major. Therefore, our data reveal that IL-4Rα-mediated mechanisms in macrophages are involved in the development of early disease progression after L. major infection. In contrast, IL-4/IL-13 responsiveness in cells different from macrophages/neutrophils appear to promote the outcome of disease in susceptible BALB/c mice during later stages of experimental leishmaniasis. Because the expression of IL-10 is impaired in CD4+ T cells from L. major-infected IL-4Rα−/− mice (2), one explanation for the transient effect of IL-4Rα deficiency in neutrophils/macrophages after L. major infection could be that the IL-4-driven production of immunosuppressive cytokines by CD4+ T cells abolished the initial protective immune responses in LysMcreIL-4Rα−/−BALB/c mice. However, we could not find significant differences between infected IL-4Rα−/−, IL-4Rα−/− and LysMcreIL-4Rα−/− BALB/c (data not shown).

IL-4- and IL-4Rα-mediated signaling have been implicated in a variety of experimental systems in the development of Th2 and type 2 Ab immune responses indicated by the development of IL-4-producing CD4+ Th2 cells and a distinct profile of Ab isotype secretion by B cells (IgG1 and IgE) (24, 25). In contrast, Th1 and type 1 Ab responses are characterized by IFN-γ-producing CD4+ Th1 cells and a different Ab isotype profile (IgG2a and IgG2b). As Th2 and type 2 Ab immune responses were originally implicated in the fatal outcome of cutaneous leishmaniasis (2), we addressed whether delayed disease progression in LysMcreIL-4Rα−/− BALB/c mice was accompanied by an altered Th1/Th2 and type1/type2 Ab immune response. Interestingly, L. major-infected LysMcreIL-4Rα−/− BALB/c mice displayed a mixed Th1/Th2 immune response. Whereas the type 2 Ab isotype profile was similar to IL-4Rα−/− BALB/c mice, lymphocytes from LysMcreIL-4Rα−/− BALB/c mice apparently developed a Th1/Th2 phenotype. A profound IL-4 response to L. major Ag was present in all mutant strains but not in C57BL/6 mice, confirming our previous data that Th2 development and the production of type 2 Abs in cutaneous leishmaniasis is independent of the IL-4Rα (7). Other factors, like IL-10 or TGF-β could also have been involved in the observed immune modulation (26, 27), even so, we did not find obvious differences during the analysis (data not shown). Increased Th1-mediated IFN-γ production in LysMcreIL-4Rα−/− BALB/c mice compared with complete IL-4Rα−/− BALB/c mice may indicate that IL-4Rα responsiveness is necessary for optimal IFN-γ production. This suggests that caMφ may promote Th1 responses as previously found in Schistosoma mansoni-infected LysMcre IL-4Rα−/− BALB/c mice (16). Because IL-4Rα engagement suppresses IL-12 production in macrophages (8), macrophage-specific IL-4Rα deficiency may uncover IL-4Rα-dependent proinflammatory mechanisms such as the IL-4-mediated instruction of dendritic cells to produce IL-12 which has been shown to promote Th1 cell development and resistance in L. major-infected BALB/c mice (28). However, we did not consistently find increased IL-12 in infected LysMcreIL-4Rα−/− BALB/c mice (data not shown). Factors produced by alternatively activated macrophages may also be directly involved in the regulation of Th1 immune responses. Accordingly, we recently identified reactive oxygen intermediates and 12/15 lipoxigenase to be suppressive molecules produced by alternatively activated myeloid cells capable of inhibiting T cell proliferation (29).

camuMφ plays an important role in combating infection with L. major through IFN-γ-induced expression of effector molecules such as NOS-2-dependent production of RNI (14–16). Importantly, inhibition of endogenous NOS-2 reactivates latent leishmaniasis in resistant mouse strains during the whole course of infection, indicating that NOS-2-dependent mechanisms in macrophages are crucial for the control of L. major persisting in immunocompetent hosts after resolution of the primary infection (30). The production of RNI is counterregulated by IL-4Rα-dependent mechanisms, leading to arginase I-expressing alternatively activated macrophages. Because NOS-2 shares L-arginine as a substrate with arginase I, substrate depletion by either enzyme is a key regulatory mechanism in macrophages and differential expression of NOS-2 and arginase I is important for regulating macrophage effector functions (17, 18). Accordingly, in contrast to macrophages from L. major-infected susceptible IL-4Rα−/− BALB/c mice, macrophages from infected resistant C57BL/6, IL-4Rα−/− and LysMcreIL-4Rα−/− BALB/c mice showed biased classical activation phenotypes, suggesting that classical macrophage effector mechanisms were enhanced in the absence of IL-4Rα responsiveness. This was directly shown after LPS restimulation as arginase activity was reduced and NO production was increased in macrophages from infected C57BL/6, IL-4Rα−/−, and LysMcreIL-4Rα−/− BALB/c mice, compared with macrophages from infected IL-4Rα−/− BALB/c mice. Although we have not directly addressed the expression of Nos-2 and arginase I, our results indicate that caMφ from IL-4Rα−/− and LysMcreIL-4Rα−/− BALB/c mice were able to efficiently express Nos-2-dependent leishmanicidal effector mechanisms in the presence of IL-4 possibly due to impaired IL-4Rα-mediated arginase I activities. In line with our findings are recent published results which postulated a role of aaMφ and arginase I expression for susceptibility to experimental L. major infection (31, 32). In the absence of endogenous Nos-2, increased resistance of BALB/c mice to infection with L. major was accompanied with a switch from alternative to caMφ (32). Vice versa, arginase I activity in alternatively activated macrophages from TLR 4-deficient C57BL/10 ScSn mice was coincident with an increased susceptibility to L. major infection (31). The most striking evidence that arginase does play an essential role in mediating susceptibility to infection with L. major came from a recent report in which neutralization of endogenous arginase with N-hydroxy-nor-L-arginine ameliorated disease progression in BALB/c mice (33). Importantly, IL-4 was shown to mediate arginase I expression and to promote elevated parasite growth in macrophages dependent on arginase activity and the arginase-dependent production of polyamines. Therefore, in our study, IL-4-mediated suppression of leishmanicidal effector mechanisms in alternatively activated macrophages from IL-4Rα−/− BALB/c mice was likely due to the induction of arginase I as originally described by Kropf et al. (33). This suppressive effect of IL-4-induced arginase activity could on the one hand lead to the reduced RNI synthesis by substrate depletion. On the other hand, IL-4-dependent production of polyamines through the enzymatic activity of arginase could directly promote parasite growth. In contrast, other studies have shown that, in the absence of LPS, IL-4 can synergize with IFN-γ for the intracellular elimination of L. major in a TNF- and RNI-dependent manner (34, 35). Different incubation protocols could have been responsible for these discrepancies. Whereas coincubation of IL-4 and IFN-γ results in a synergistic induction of RNI (34, 35), preincubation of IL-4 down-regulates NO production (8, 36). In contrast to the mentioned in vitro studies, LysMcreIL-4Rα−/− BALB/c mice enabled us to analyze the impact of IL-4Rα-mediated signaling in macrophages on the outcome of experimental leishmaniasis in vivo. Our studies revealed that, after infection with L. major, a switch from aaMφ to caMφ results in an increased production of RNI followed by a delayed
disease progression in LysMcreIL-4R+/− BALB/c mice. A similar increased production of RNI accompanied with an impairment in aaM and subsequent arginase I activity is, as our study showed, rather of advantage for the parasite and may be part of its evolved evasion mechanisms. This hypothesis is also substantiated by the fact that Leishmania itself expresses endogenous arginase I, which plays a pivotal role in polyamine precursor metabolism and is essential for parasite survival as recently shown using arginase I-deficient parasites (40). Second, it has been demonstrated that the parasite is able to exploit the host’s IL-4R-dependent arginase I-mediated polyamine metabolism (33). Overall, our data show evidence that aaM is disadvantageous for host protection against leishmaniasis, as it prevents efficient Th1 and type 1 Ab responses and suppresses macrophage effector functions. However, aam can also modulate Th1-type responses to prevent excessive inflammation to ensure host survival during infection as such schistosomiasis (8). In conclusion, aaM limits the pathological sequelae of excessive inflammation but may also prevent optimal antimicrobial protection.

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Disclosures

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