Enhanced Susceptibility to *Leishmania* Infection in Resistant Mice in the Absence of Immediate Early Response Gene X-1

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Enhanced Susceptibility to *Leishmania* Infection in Resistant Mice in the Absence of Immediate Early Response Gene X-1

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Immediate early response gene X-1 (IEX-1) is a stress-inducible gene abundantly expressed in macrophages and T cells following various stimuli. To explore a potential role for IEX-1 in control of the susceptibility to *Leishmania major* infection, the inflammatory response during cutaneous leishmaniasis was evaluated in 129Sv/C57BL6-resistant mice in the presence or absence of IEX-1. Null mutation of IEX-1 enhanced the susceptibility of the mice to *L. major* infection, and aggravated inflammatory responses in comparison with wild-type control mice. The excessive inflammation was not ascribed to a Th2-biased immune response or a defect in Th1 polarization, but rather to an elevated level of IL-17 production by both γδ T and CD4+ cells, concomitant with an increase of the neutrophil recruitment early in the infection. The lack of IEX-1 also suppressed TNF-α production in both macrophages and T cells, resulting in a high intralesional load of parasites and delayed healing of the lesion, both of which were reversed by TNF-α treatment. These findings indicate the crucial role of IL-17 and TNF-α in determining the outcome of *L. major* infection beyond a balance between Th1- and Th2-mediated immune responses. *The Journal of Immunology*, 2009, 183: 7994–8003.

*Leishmania major* is an intracellular protozoan parasite of macrophages and a cause of a wide range of cutaneous granulomatous diseases in humans. Macrophages serve not only as the host cells for the replication of parasites, but they also act as effector cells that are responsible for parasitic killing after activation by cytokines released from Th1 cells (1, 2). The resolution of leishmanial infections requires the expansion of specific Th1 cells that secrete predominantly IFN-γ, which is capable of activating macrophages (3). On the contrary, development of IL-4-driven Th2 cells exacerbates the disease as a result of macrophage deactivation (4). Therefore, cutaneous leishmaniasis (CL) became the model of choice to study the dichotomy between the Th1 and Th2 immune responses in mice. Recently, the simplicity of this model has been challenged by studies with genetically manipulated mice. These studies reveal more complexities in the mechanisms of mouse susceptibility to *L. major* infection. For instance, infection with *L. major* was not controlled at all or only partially controlled in IL-4-deficient mice depending on the *L. major* strain of the infection, even though IFN-γ production and response were intact (5). In *L. major*-susceptible BALB/c mice, the expression of a dominant-negative form of TGF-β receptor type II in T cells delayed the formation of *Leishmania*-induced lesions and slowed down the lesion progression, despite the fact that the mice maintained a strong Th2-mediated response (6). Moreover, *L. major* infection was exacerbated by treatment with TGF-β in C57BL6/BALB/c F1 mice, whereas treatment with anti-TGF-β Ab enhanced the resistance of these mice to the infection (7). The compromised effect of TGF-β on *L. major* infection hints that T cells producing IL-17 may contribute to the complex immune response in *L. major* infection in light of the essential role of TGF-β in the differentiation of IL-17-producing T cells (8–10).

The immediate early response gene X-1 (IEX-1) is a stress-inducible gene. It is abundantly expressed in macrophages and T cells following various stimuli. IEX-1 can affect the proliferation, differentiation, and survival of these cells due to its role in the modulation of mitochondrial oxidative phosphorylation and the production of reactive oxygen species (ROS) by targeting the mitochondrial F1Fo-ATPase inhibitor to degradation (11). Overexpression of IEX-1 leads to increased survival of activated T cells, predisposing to a lupus-like autoimmune disease in mice transgenic-expressing IEX-1 in the lymphocytes under the control of the Ig H chain (μ) enhancer (12).

To explore a potential role for IEX-1 in controlling the susceptibility of *L. major* infection, the inflammatory response in resistant mice was evaluated in the present study following *L. major* infection in the presence or absence of IEX-1. IEX-1 knockout (KO) mice displayed enhanced susceptibility to *L. major* infection and developed an aggravated inflammatory response in comparison with wild-type (WT) mice. Interestingly, the aggravated inflammation was not ascribed to enhanced Th2-mediated immune response or a defect in the Th1-mediated response, but rather to an increased production of IL-17 by γδ T cells and Th17 cells, concomitant with the neutrophil infiltration early in the process. In addition to the enhanced IL-17 production by γδ T cells and Th17 cells, the aberrant response of IEX-1 KO macrophages to IFN-γ as a result of impaired TNF-α production also contributed to the high intralesional load of parasites and delayed healing of the lesion. These findings indicate that IEX-1 takes part in the shaping of macrophagal activation and in the regulation of IL-17 and TNF-α production during *L. major* infection. IL-17-producing T cells may...
be as important as Th1 and Th2 cells in determining the outcome of *L. major* infection.

**Materials and Methods**

**Parasites**

The *L. major* National Institutes of Health Friedlin V1 strain (MHOM/IL/80/AFN), isolated from a patient with localized CL in Israel, was a gift of M. McDowell (Department of Biological Science, University of Notre Dame, Notre Dame, IN). *Leishmania* parasites were cultured at 24°C without CO2 in medium 199 (M199) supplemented with 20% heat-inactivated FCS, 100 μg/ml streptomycin, 2 mM L-glutamine, 40 mM HEPEs, 0.1 mM adenine (in 50 mM HEPEs), 5 mg/ml hemin (in 50% triethanolamine), and 1 mg/ml 6-biotin (medium 199 complete (M199-C)). Infective-stage metacyclic promastigotes were isolated from stationary cultures (4–5 days old) by using a uniform procedure based on a modification of a method of density gradient purification (13). Briefly, 10 ml of parasite suspension in M199-C containing ~1 × 10⁶ stationary-phase promastigotes was layered on a discontinuous density gradient in a 50-ml conical Falcon tube consisting of 10 ml of 20% Ficoll stock solution made in distilled water and 10 ml of 10% Ficoll diluted in M199-C. The gradient was centrifuged for 15 min at 2000 × g at room temperature, and the parasites in the upper 10% Ficoll were collected and washed by centrifugation at 3000 × g.

**Animals and infection**

IEX-1 KO and WT control mice on the mixed 129Sv/C57BL/6 background were generated by gene targeting (M. Shahid, L. Shen, I. Ustyugova, D. Seldin, B. Lu, X. Chen, W. Zapol, and M. X. Wu, manuscript in preparation) (28). Mice were housed in conventional cages at the animal facilities of Massachusetts General Hospital in compliance with institutional guidelines. The Institutional Animal Care and Use Committee for Massachusetts General Hospital approved all animal experiments. For in vivo infections, 1 × 10⁶ metacyclic parasites in 20 μl of PBS were inoculated intradermally into ears of 4- to 6-wk-old female mice using a 27.5-gauge needle. The evolution of the lesion was monitored for 11 wk. The diameters of the lesions were measured using a dial gauge Vernier caliper.

**Quantification of parasite loads**

Parasite loads in the ears were determined, as described previously (14). In brief, the two sheets of the ear lobe were separated, placed dermal side down in DMEM containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mg/ml collagenase A (Sigma-Aldrich), and incubated for 1 h at 37°C. The sheets were then cut into small pieces in 6-well plates containing 2 ml of M199 without FCS. The tissue homogenates were filtered using a 70-μm nylon cell strainer before being washed twice in RPMI 1640, NaHCO3, penicillin/streptomycin/gen-tamicin, containing 125 U/ml penicillin (Sigma-Aldrich) for 2 h. The dermal sheets from three to five animals were pooled, cut into small pieces, and filtered through a 40-μm nylon cell strainer before being washed twice in RPMI 1640, NaHCO3, penicillin/streptomycin/gentamicin, 10% FCS, and 0.05% DNase I (Sigma-Aldrich).

**Flow cytometry**

Cells were harvested from the regional lymph nodes, washed twice with PBS, and incubated with 10% normal serum to block unspecific binding. Cells were then stained and analyzed in parallel as a positive control (BD Biosciences). Negative control was the cells incubated with an irrelevant Ab (A95-1, PE-conjugated rat IgG2b; BD Pharmingen). We used FITC anti-mouse CD4 (RM4-5) and PE anti-mouse CD25 (PC61.5) from eBioscience, in combination with Alexa Fluor 647 anti-Foxp3 Ab (BioLegend) to stain T regulatory (Treg) cells in a Foxp3-FITC/PE Ch3 (BioLegend) buffer set (BD Biosciences). PE hamster anti-mouse y8 TCR Ab (GL3) was used with FITC hamster anti-mouse CD3ε (145-2C11) for y8 T cell detection. The cells were evaluated using FACSAria and CellQuest software (BD Biosciences). FlowJo software (Tree Star) was used for analysis of flow cytometric data.

**Cytometric bead array (CBA)**

The level of cytokines (IL-6, IL-10, MCP-1 IFN-γ, TNF-α, and IL-12p70) was measured in the homogenate of the CL lesion by a BD Mouse Inflammation CBA kit (BD Biosciences). The assays were performed, according to the manufacturer’s instructions. In brief, murine ears with the CL lesions were removed immediately after euthanasia and placed in 70% ethanol for 2 min for sterilization of the external surfaces of the ears. Then, the whole ear was transferred into 5-ml glass tissue grinder containing 2 ml of RPMI 1640 medium and homogenized, followed by centrifugation for 15 min at 3500 × g at room temperature. The resultant supernatant was collected and stored at −80°C until analysis. A 50-μl aliquot of the supernatant was stained with a mixture of mouse cytokine capture bead suspension and PE detection reagent. After 2 h of incubation, samples were washed and analyzed by the BD CBA software. Mouse inflammation cytokine standards provided with the kit were appropriately diluted and measured in parallel as standards.

**ELISA**

The concentration of TNF-α or IL-17A in the tissue supernatant and medium from the in vitro experiment was detected by ELISA Ready-Set-Go! according to the manufacturer’s protocol (eBioscience).
INFECTION IN IEX-1 KO MICE

Leishmania infection in IEX-1 KO mice

To determine the immune alteration in association with the aggravated inflammation in IEX-1 KO mice, we evaluated the percentage of CD4+CD25+Foxp3+ cells in the draining lymph nodes from these mice 5 days after L. major infection. Treg cells are crucial to the host parasite interaction, controlling the extent of the immune-mediated pathology (16). We found that the percentage of Treg cells in IEX-1 KO mice was twice less than in WT control mice (p < 0.01; Fig. 2A). A decrease in the level of Treg cells correlated with the aggravated inflammation induced by L. major infection observed in IEX-1 KO mice, which was expected to enhance either Th1- or Th2-mediated immune responses (17). However, this was not the case. When the levels of T cells producing IFN-γ or IL-4 were evaluated, we found that both groups of mice had similar percentages of IFN-γ-producing CD4+ cells provoked by the infection (Fig. 2B). There was no significant difference either in the number of IFN-γ-producing CD4+ cells between WT and IEX-1 KO mice (Fig. 2C). The levels of IL-12, IFN-γ, MCP-1, and IL-6 in the skin with the CL lesions were also comparable in the presence or absence of IEX-1 (Fig. 2D). A decrease in the level of TNF-α and an increase in IL-10 production were evident in IEX-1 KO lesions (Fig. 2D). Neither the number of IL-4-producing cells (Fig. 2B) nor the IL-4 level was increased (data not shown), ruling out the possibility that an enhanced Th2-mediated response contributed to the aggravated inflammatory response in the absence of IEX-1.

Increased production of IL-17A in the CL lesion of IEX-1 KO mice

We next investigated possible contribution of IL-17 to the observed difference in L. major-induced inflammation in the presence or absence of IEX-1, in light of its highly proinflammatory nature and the reciprocal differentiation between Th17 cells and Treg cells. It was found that the level of IL-17 (IL-17A) was 2-fold higher in the inflamed tissues of IEX-1 KO mice than of WT control mice (Fig. 2D). To pinpoint the T cells involved, IL-17-producing T cells were analyzed in the draining lymph nodes of infected IEX-1 KO and WT control mice. In accordance with increasing levels of IL-17 in the lesions, the percentage of IL-17-positive lymphocytes was significantly higher in IEX-1 KO mice than in WT control mice 5 days, but not 9 wk, after infection (Fig. 3A). Both CD4+ T cells and γδ T cells producing IL-17 were increased significantly 5 days after infection (Fig. 3B and C), although only γδ T cells producing IL-17 were persistently elevated in the later stage of the infection in the absence of IEX-1 (Fig. 3C). The data conclude that the increased level of IL-17 in the CL lesion was ascribed to both Th17 and γδ T cells.

One of the key functions of IL-17 is the recruitment of neutrophils to the site of inflammation. Indeed, accumulation of inflammatory cells largely composed of neutrophils was observed in the infected areas lacking IEX-1 (Fig. 3E). The percentage of granulocytes was 4 times higher in IEX-1 KO mice than in the WT mice infected similarly (Fig. 3E). The increase in the number of neutrophils was confirmed by the myeloperoxidase activity assay (Fig. 3F). These findings were in keeping with histological pictures (data not shown). The inflammatory infiltrate in IEX-1 KO mice}
consisted of a mixture of macrophages with a significant number of polymorphonuclear neutrophils and lymphocytes. In comparison, the early CL lesions in WT mice were characterized by numerous macrophages with few eosinophils.

Macrophages from IEX-1 KO mice exhibit aberrant responses to L. major infection

Macrophages play a key role in both Leishmania survival and eradication (2). IEX-1 is highly expressed in macrophages, and its absence may alter the response of macrophages to L. major infection, contributing to the aggravated inflammation in IEX-1 KO mice (Fig. 1). This was corroborated by a 100-fold increase in the parasite load in the infected ears of IEX-1 KO mice relative to WT control mice (Fig. 4A). The increased parasite load may have resulted from the augmented engulfment of parasites by the macrophages and/or their enhanced replication, or from a defect in the parasite killing by the activated macrophages. To investigate these possibilities, we first examined the infection and the replication rate of parasites in macrophages in the presence or absence of IEX-1.

Macrophages were derived from the bone marrow (BMDM) of WT and IEX-1 KO mice, and subsequently infected with L. major promastigotes at a macrophage to promastigote ratio of 1:5. The number of amastigotes was counted at 2 and 24 h after infection to determine the dynamics of the parasite engulfment and the replication rates, respectively. We found a 60% decrease in the number of intracellular amastigotes per 100 cells at 2 h after infection or by a 65% decrease at 24 h, respectively, in IEX-1 KO macrophages compared with WT counterparts (Fig. 4B).
Under a microscope, densely packaged amastigotes were seen in WT cells, in contrast to a sparse number observed in IEX-1 KO macrophages (Fig. 4C). It is unlikely that this defect in IEX-1-deficient macrophages is due to a decrease in the expression of the surface receptors, because the levels of CR3, FcγIII/II, CD204, or CD206 molecules that are known to be involved in adhesion and the invasion of *L. major* to macrophages were comparable between the IEX-1 KO and WT macrophages (data not shown). In support, no significant difference was observed in the number of attached nonopsonized *Leishmania* parasites to BMDM at 2 and 24 h after coincubation. The significant decrease in the number of intracellular amastigotes without concurrence of detectable alteration in the number of adherent, nonopsonized *Leishmania* parasites argues strongly that lack of IEX-1 hinders the entrance of *Leishmania* parasites into the host cells.

We went on to assess the IFN-γ response of macrophages in the presence or absence of IEX-1, which plays a key role in the control of the replication of *L. major* amastigotes (18, 19). BMDM from WT and IEX-1 KO mice were preincubated with IFN-γ for 4 h before *L. major* infection, and the number of amastigotes per 100 cells was determined 24 h later. Pretreatment with IFN-γ resulted in a 2-fold decrease in the number of amastigotes in WT macrophages.

FIGURE 3. Increased IL-17A production and neutrophil infiltration in IEX-1 KO mice. A–C, An increased number of IL-17A-producing CD4 and γδ T cells in IEX-1 KO mice. The percentages of IL-17A⁺CD4⁺ lymphocytes from submandibular drain lymph nodes were analyzed, as described in Materials and Methods. Representative flow cytometric profiles are shown in A. The percentages of CD4⁺IL-17A⁺ cells and IL-17A⁺γδ T cells are shown in B and C, respectively. *, *p < 0.05 and **, *p < 0.01. D, Increased levels of IL-17A in the CL lesions in IEX-1 KO mice 5 days after *L. major* infection. IL-17A in the homogenate of the CL lesions was measured by ELISA (*n* = 10; *, *p < 0.05). E and F, Increased numbers and activity of granulocytes in the CL lesion in IEX-1 KO mice. E, Percentages of Ly-6G/Ly-6C⁺ granulocytes relative to total cells of the CL lesion were analyzed by flow cytometry in 5 days after *L. major* infection (*n* = 10). F, The supernatant derived from homogenates of the CL lesions 5 days after infection was assayed for myeloperoxidase activity (*n* = 10).
IFN-γ WT cells in response to IFN-γ were infected, they produced significantly less nitrite than infected macrophages. The burden was previously demonstrated in IFN-γ-macrophages. With or without reports (23, 24), WT BMDM stimulated with IFN-γ infected macrocytes by quantification of nitrite (NO₂⁻) production in infected macrophages (20–22). We measured the NO production of IEX-1 KO macrophages, irrespective of IEX-1 expression. Taken together, these data suggest that IEX-1 KO macrophages have a reduced response to IFN-γ after infection with L. major, reducing nitrite production.

**FIGURE 4.** Aberrant responses of IEX-1 KO macrophages to L. major infection. A. Increased numbers of parasites in IEX-1 KO mice compared with WT mice. The parasitic load in the CL lesions was quantified 7 wk after infection by a standard dilution assay. Each dot represents data from individual samples. ***, p < 0.01. B and C. The level of parasite engulfment is decreased in IEX-1 KO relative to WT BMDM. WT and IEX-1 KO BMDM were infected with L. major metacyclic promastigotes at a ratio of BMDM to parasites of 1:5. The numbers of parasites per 100 cells were assessed by light microscopy in 2 and 24 h. ***, p < 0.01; ***, p < 0.001. C. IEX-1-deficient BMDM contains less amastigotes 24 h after infection. Diff-Quik staining (×1000). D. Pretreatment with IFN-γ does not enhance an intracellular destruction of leishmanial parasites by IEX-1 KO BMDM. BMDM from WT and IEX-1 KO mice were preincubated with 10 ng/ml IFN-γ for 4 h before L. major infection. The number of amastigotes per 100 cells was determined as in B in 24 h. ***, p < 0.001, in comparison with no IFN-γ; ††, p < 0.01 in comparison with WT. E. The IFN-γ-induced NO production is reduced in the absence of IEX-1. WT or IEX-1 KO BMDM were infected with or without L. major, as above, for 48 h in the presence or absence of varying concentrations of IFN-γ. NO₂⁻ in the resultant culture medium was measured by Griess reaction. Data are shown as mean ± SD. *, p < 0.05; ***, p < 0.01; and ***, p < 0.001.

(p < 0.001), whereas the number of amastigotes remained unchanged in IEX-1 KO macrophages, whether or not the cells were treated with IFN-γ (Fig. 4D). These results clearly show that lack of IEX-1 impairs IFN-γ-induced leishmanicidal activity in macrophages.

The importance of NO production in controlling the parasitic burden was previously demonstrated in IFN-γ-treated L. major-infected macrophages (20–22). We measured the NO production in infected macrophages by quantification of nitrite (NO₂⁻), which is one of two primary, stable, and nonvolatile breakdown products of NO. We compared the ability of WT and IEX-1 KO BMDM to produce NO after stimulation by IFN-γ. Consistent with previous reports (23, 24), WT BMDM stimulated with IFN-γ produced nitrite in a dose-dependent manner, with a ~56-fold increase in infected compared with uninfected macrophages (Fig. 4E). In comparison with noninfected WT cells, noninfected IEX-1 KO BMDM were much more responsive to IFN-γ stimulation, producing 4 to 6 times more nitrates. The basal level of NO production was also higher in IEX-1 KO mice (p < 0.01). However, when the cells were infected, they produced significantly less nitrite than infected WT cells in response to IFN-γ. Despite a reduced level of nitrite production in infected, IEX-1 KO macrophages, NO₂⁻ was produced at a considerably high level in infected compared with uninfected BMDM, irrespective of IEX-1 expression. Taken together, these data suggest that IEX-1 KO macrophages have a reduced response to IFN-γ after infection with L. major, reducing nitrite production.

**TNF-α production is down-regulated in IEX-1 KO mice**

In addition to the aggrandized inflammation induced by L. major infection, a delay in the healing process by 3–4 wk was another discernible feature of lesions in the absence of IEX-1. This prompted us to test the level of TNF-α in the infected ears, because neutralization of TNF-α in resistant mice led to a delay in the healing process (25). Moreover, TNF-α KO mice infected with L. major developed nonhealing lesions, characterized by an elevated presence of neutrophils at the site of infection and only partial control of parasite numbers within the lesions (26). As shown in Fig. 5A, a significantly low level of TNF-α production was found in the CL lesions of IEX-1 KO mice compared with WT control mice infected similarly for 5 days (p < 0.001), and to a lesser degree, for 7 wk (p < 0.01). Although the level of TNF-α in WT decreased gradually during the course of infection, TNF-α remained low in IEX-1-KO mice during the same time. The low level of TNF-α production resulted from a defect in both macrophages and T cells in the absence of IEX-1. In comparison with WT macrophages, IEX-1-deficient macrophages had a similar basal level of TNF-α production, but a reduced level in response to IFN-γ stimulation (Fig. 5B). Moreover, in contrast to induction of TNF-α by L. major infection in WT macrophages, the infection did not significantly induce TNF-α production over uninfected, IEX-1-deficient macrophages. Although a combination of L. major infection and IFN-γ augmented TNF-α production significantly in both KO and WT macrophages, the degree of the increase was...
much smaller in IEX-1 KO cells than in WT cells (Fig. 5B). Likewise, the percentage of TNF-α-positive CD4⁺ cells was 1.5 times less in IEX-1 KO mice than in WT mice (Fig. 5C). Although 72% of WT CD4⁺ cells produced TNF-α, only 53% of IEX-1 KO CD4⁺ cells were capable of producing this cytokine (Fig. 5D). Thus, the delay of healing in IEX-1 KO mice may be ascribed to the diminished production of TNF-α in both macrophages and T cells.

**Discussion**

Our present study demonstrates that lack of IEX-1 alters the function of both macrophages and T cells, resulting in the enhanced susceptibility to *L. major* infection in resistant mice independent of an enhanced Th2 immune response. IEX-1 deficiency did not cause an increase in the number of IL-4-secreted Th2 cells or a decrease in IFN-γ-produced Th1 cells. Rather, lack of IEX-1 augmented IL-17 production by both γδ T cells and Th17 cells in the early phase of infection and predominantly by γδ T cells in the late phase, concurrent with neutrophil infiltration and persistent inflammation in the infected site. IEX-1 KO mice thus represent a novel model of susceptibility to *L. major* infection.
model to study the complex immune response that extends beyond a balance between Th1 and Th2 cells. Absence of IEX-1 also impairs TNF-α production in both T cells and macrophages following *L. major* infection, which is associated with a significant delay in lesion resolution and reduced IFN-γ responses in *L. major*-infected macrophages. Apparently, in addition to the indispensable role of IEX-1 in the regulation of T cell differentiation, survival, and vascular activity (12, 28, 29), IEX-1 is pivotal in macrophage activation and in macrophagal resistance to intracellular parasites such as *L. major*.

The balance between Th1- and Th2-mediated immune responses has been long believed to be a key factor in determining the outcome of *L. major* infection. But, recent progress made in elucidation of Treg and Th17 cells has challenged this paradigm. Treg cells were shown to control the severity of an inflammatory response by preserving surrounding tissue from unnecessary damage during an early stage of CL (30, 31). The mechanism of this suppression may involve impact of Treg cells on Th1/Th2 dichotomy during the immune recognition, although the findings in support of this theory are controversial (17, 32, 33).

A decrease in the percentage of Treg cells was also not related to a Th2-biased immune response or a defect in Th1 polarization in IEX-1 KO mice. Our data suggest that IL-17 secreted by both y6 T cells and Th17 cells can aggravate the inflammation and infection of *L. major* in the absence of a Th2-skewed immune response. This finding is consistent with the deteriorated role of TGF-β in *L. major* infection, previously demonstrated in various animal models (6, 7), and the persistence of neutrophils in susceptible – but not in resistant – mouse strains (34). Consistent with the prominent role of IL-17 in neutrophil infiltration (35), a high number of neutrophils was seen in the lesions in IEX-1 KO mice compared with WT mice after infection. The results mirror recent findings that IL-17-deficient mice displayed a decrease in neutrophil immigration into lesion after *L. major* infection, concomitant with a diminished lesion volume and a low parasite burden (10). These studies stress that an increase in IL-17-dependent neutrophil recruitment into *L. major* lesions significantly contributes to the determination of the disease outcome (36, 37).

IFN-γ induces NO and superoxide production, leading to peroxidation of amastigote membranes and the subsequent death of
intracellular parasites (21). Several lines of evidence indicate that the capability of IFN-γ to activate inducible NO synthase (iNOS) in infected macrophages is dependent on TNF-α (19, 27). Mice resistant to infection with L. major have elevated levels of TNF-α in their draining lymph nodes, whereas no TNF-α is detectable in susceptible animals during the course of the infection (38). Moreover, anti-TNF-α treatment blocked intracellular killing of leishmanial parasites by IFN-γ-treated macrophages in a dose-dependent fashion (39). Anti-TNF-α Ab was also found abrogating the NO production in IFN-γ-treated macrophages infected with amastigotes, suggesting that TNF-α is a downstream signal in IFN-γ-mediated activation of iNOS. Our data confirm the contributive role of TNF-α in IFN-γ-mediated activation of iNOS production. The absence of IEX-1 goesets, and reduced IFN-α in L. major macrophostes in a dose-dependent fashion (39). Anti-TNF-α Ab would result in NF-κB to the promoter is dependent on reduc-

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References


The online version of this article contains supplemental material.


