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Mice Deficient in LRG-47 Display Enhanced Susceptibility to Trypanosoma cruzi Infection Associated with Defective Hemopoiesis and Intracellular Control of Parasite Growth

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IFN-γ is known to be required for host control of intracellular Trypanosoma cruzi infection in mice, although the basis of its protective function is poorly understood. LRG-47 is an IFN-inducible p47GTPase that has been shown to regulate host resistance to intracellular pathogens. To investigate the possible role of LRG-47 in IFN-γ-dependent control of T. cruzi infection, LRG-47 knockout (KO) and wild-type (WT) mice were infected with the Y strain of this parasite, and host responses were analyzed. When assayed on day 12 after parasite inoculation, LRG-47 KO mice, in contrast to IFN-γ knocko ut (KO) and wild-type (WT) mice, were infected with the Y strain of this parasite, and host responses were analyzed. When assayed on day 12 after parasite inoculation, LRG-47 KO mice, in contrast to IFN-γ KO mice, controlled early parasitemia almost as effectively as WT animals. However, the infected LRG-47 KO mice displayed a rebound in parasite growth on day 15, and all succumbed to the infection by day 19. Additional analysis indicated that LRG-47-deficient mice exhibit unimpaired proinflammatory responses throughout the infection. Instead, reactivated disease in the KO animals was associated with severe splenic and thymic atrophy, anemia, and thrombocytopenia not observed in their WT counterparts. In addition, in vitro studies revealed that IFN-γ-stimulated LRG-47 KO macrophages display defective intracellular killing of amastigotes despite normal expression of TNF and NO synthetase type 2 and that both NO synthetase type 2 and LRG-47 are required for optimum IFN-γ-dependent restriction of parasite growth. Together, these data establish that LRG-47 can influence pathogen control by simultaneously regulating macrophage-microbicidal activity and hemopoietic function. The Journal of Immunology, 2005, 175: 8165–8172.

Interferon-γ is a key mediator of both innate and acquired immunity to pathogens. In phagocytic cells this cytokine promotes intracellular killing of microbes through the induction of toxic metabolites, such as reactive oxygen species and NO, and also up-regulates the expression of class I and class II MHC molecules, thereby stimulating Ag presentation to T cells (1). In addition, IFN-γ induces other nonhemopoietic cell types, such as endothelial cells, keratinocytes, and fibroblasts, to secrete a wide variety of different proinflammatory mediators. Finally, IFN-γ has been shown to play a role as a negative regulator of lymphocyte expansion, preventing uncontrolled lymphoproliferation during the response to infection (1). Nevertheless, a complex program of >1200 genes is induced by IFN-γ in host cells, and the mechanisms by which these functions are integrated to mediate host resistance to infectious challenge in vivo are only partially understood (2).

Recently, a new group of IFN-γ-induced genes has been identified that plays a major role in host control of intracellular pathogens (3, 4). These genes belong to a family encoding a series of 47- to 48-kDa GTPases (5). At present, six proteins have been described in the mouse: inducibly expressed GTPase (IGTP),2 LRG-47, IRG-47, TGTP/Mg21, IIGP, and GTPI (3, 4, 6). These GTPases are expressed by a variety of cell types in response to stimulation by both type I and type II IFNs. Such widespread inducibility may allow the molecules to mediate cell-autonomous effects against pathogens. Indeed, mice deficient in different p47GTPases display acute susceptibility to a wide variety of intracellular protozoa and bacteria. This ablation in host resistance depends on the particular GTPase knockout (KO) and pathogen combination studied (3). IGTP KO mice, for example, are highly susceptible to the protozoan pathogens Toxoplasma gondii (7) and Leishmania major (Y. Belkaid, unpublished observations), but are resistant to Trypanosoma cruzi (8) and all intracellular bacteria examined to date (7, 9–11). In contrast, LRG-47 KO mice are extremely susceptible to Trypanosoma gondii (7) and L. major (Y. Belkaid, unpublished observations) as well as a large number of different bacteria, including Listeria (7), Mycobacteria (4, 11), and Salmonella (G. Taylor, unpublished observations). Because of its profound and broad effects on host resistance to intracellular pathogens, most recent studies of the protective role of p47GTPases have focused on LRG-47.

2 Abbreviations used in this paper: IGTP, inducibly expressed GTPase; BMMø, bone marrow-derived macrophage; KO, knockout; L-NMMA, Nω-monomethyl-L-arginine acetate; MOI, multiplicity of infection; NOS2, NO synthetase type 2; WT, wild type.

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LRG-47 is a membrane-associated protein that in resting cells is localized to the Golgi by a C-terminal amphipathic helix (12). During phagocytosis, LRG-47 is recruited to the plasma membrane where it becomes associated with phagocytic cups (12) and in a recent study was shown to play an important role in accelerating phagosome maturation and lysosome-phagosome fusion (10). This function was demonstrated to be necessary for the control of *Mycobacterium tuberculosis* killing by IFN-γ-activated macrophages and was reflected in the increased susceptibility and acute mortality of *M. tuberculosis*-infected LRG-47 KO mice (10). More recently, LRG-47 has also been shown to promote autophagy, another mechanism implicated in host resistance against intracellular microbes (13).

In addition to its role in promoting IFN-γ-dependent control of intracellular pathogen growth, LRG-47 has been shown to play a major role in regulating lymphocyte numbers. This function was revealed in a study in which LRG-47 KO mice were infected with *Mycobacterium avium*, a pathogen that is less virulent than *M. tuberculosis* and that allows chronic infection even in IFN-γ-deficient hosts. Before succumbing during early chronic infection, *M. avium*-infected LRG-47 KO mice underwent profound lymphopenia, as evidenced by reduced CD4+ and CD8+ T cell counts in spleen, thymus, and peripheral blood. The resulting loss in effector lymphocytes was proposed as a possible explanation for the increased susceptibility of these animals (11).

In the present study we have examined the role of LRG-47 in host resistance to the protozoan parasite *Trypanosoma*. This pathogen was considered of special interest for the following reasons. First, although in common with *T. gondii*, *T. cruzi* infects multiple host cell types and induces IFN-γ-dependent resistance, IGTP-deficient mice are, nevertheless, resistant to the latter parasite (8). Also in contrast to *T. gondii*, *L. major*, and mycobacteria, which reside intracellularly in plasma membrane-derived vacuoles, *T. cruzi* escapes into the cytoplasm soon after cell invasion (14, 15). Secondly, because *T. cruzi* infection can trigger transient lymphopenia followed by lymphocytosis and anemia in wild-type (WT) mice (16), it was of interest to determine whether such changes in the lymphoid compartment would be exaggerated in LRG-47-deficient mice, as previously observed by us during *M. avium* infection (11). Finally, in contrast to *M. avium*, killing of *T. cruzi* by IFN-γ-activated macrophages can be readily measured in vitro. Thus, it was thought that *T. cruzi* infection might offer the ability to simultaneously study the effects of LRG-47 on both macrophage restriction of pathogen growth as well as lymphocyte dynamics. Indeed, as described below, LRG-47 KO mice infected with *T. cruzi* displayed defects in both these functions, which were closely associated with enhanced susceptibility of the animals to parasitic infection.

### Materials and Methods

#### Mice

LRG-47 KO mice on a C57BL/6J × 129 mice background were generated as described previously (7). IFN-γ KO mice were purchased from The Jackson Laboratory. WT B6129F1 and NO synthetase type 2 (NOS2)-deficient (Y strain) was maintained by weekly passage in mice. For in vivo infection, parasites were removed by repeated (three to four times) washing with RPMI 1640, and the cells were incubated at 37°C in 5% CO2 with or without additional IFN-γ. The infected cells were then washed, fixed, and stained using a Diff-Quick kit (Dade Behring) 1 or 48 h later. To evaluate parasite growth, intracellular parasites were counted (at ×40 magnification) under a light microscope in at least 300 cells. Blinded counting confirmation was performed for all experiments. In some experiments, cells were also treated with 1 mM N6-monomethyl-L-arginine (L-NMMA; Tocris Cookson) to inhibit NOS2 activity.

#### lrg-47 gene silencing

To inhibit lrg-47 gene expression, BMMds (2 × 105 cells/well) were transfected with a commercially prepared gripNA antisense sequence according to the manufacturer’s Active Motif and as described previously (20). Briefly, cells were transfected with 3 μM FITC-labeled gripNA-murine LRG-47 (5′-ACTGTTGTAATGTTCTCAC-3′) or an equivalently labeled human CREB as a nonspecific sequence control using Charrier II as a transfection reagent (Active Motif). A predetermined concentration of 3 μM gripNA LRG-47 was used based on previous dose-response studies (A. Babca, unpublished observations). The transfected cells were then left untreated or were infected with *T. cruzi* in the absence or presence of IFN-γ for 48 h, and parasite growth was analyzed as described above. Inhibition of LRG-47 protein expression resulting from gripNA silencing was confirmed in cells infected and/or IFN-γ-stimulated for 6 h by Western blotting using a polyclonal rabbit serum generated against a unique peptide in the sequence of the protein as described by us previously (7).

#### Flow cytometry and differential cell counting

Single-cell suspensions of spleen, bone marrow, thymus, and lymph node from individual mice were prepared, and live cells were counted by trypsin blue exclusion. After adjustment of the cell concentration, cells were surface stained with mAb as previously described (11). All mAb used were obtained from BD Pharmingen. Data were collected using a FACScalibur (BD Biosciences) with CellQuest (BD Biosciences) and analyzed using FlowJo (TreeStar) software.

#### Flow cytometry and differential cell counting

RBC, platelet, and differential white blood cell counts were assayed in EDTA-treated blood using an Abbott CELL-DYN automated analyzer. The baseline ranges of blood cell counts for normal adult mice of the three WT strains used were obtained from the website of The Jackson Laboratory (http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn = docs/home) and combined to demarcate the normal limits.

#### Measurement of mRNA expression by real-time RT-PCR

Total RNA was isolated from spleens or macrophage cultures and real-time RT-PCR was performed on an ABI PRISM 7900 real-time PCR Detection system (Applied Biosystems) after RT of 1 μg RNA using SuperScript II reverse transcriptase (Invitrogen Life Technologies). The relative level of gene expression was determined by the comparative threshold cycle method as described by the manufacturer, whereby data for each sample were normalized to *hypoxanthine phosphoribosyltransferase* and expressed as a fold change compared with untreated or uninfected controls. The following primer pairs were used: for *hypoxanthine phosphoribosyltransferase*, CTGGTCCAGAGTGTGTTGTG (forward) and CAGGGCCAGACCTTGTGCAC (reverse); for IFN-γ, AGAGCCAGATTATCTTTCTTACCTCA (forward) and CTTTCTTGCCTGTCGTCTG (reverse); for nos2, TGCCCCCTCAATGTTGGA (forward) and ACTGGAGGAGCACCCCAATAT (reverse); for *inf*, AAATTTCCAGTAATGACACGGCTTATGAG (forward) and CCCTTGGAAGAGAAACTCTGGAATTGAG (reverse); for il-10, GCTTGGACAGCTATCCCGG (forward) and ACCGGCTTCACCGCTTTGCT (reverse); and for lrg-47, TGAGCTAGCAGCCTCCCTT (forward) and TGGGCAATGGTTGGCACAGT (reverse).
Quantification of parasite tissue loads by real-time PCR

Real-time PCR for parasite quantification was performed as described previously (21) with minor modifications. Briefly, on different days after infection, heart, spleen, and liver were digested with proteinase K, followed by a phenol-chloroform-isooamyl alcohol affinity extraction. Real-time PCR using 50 ng of total DNA was performed on an ABI PRISM 7900 sequence detection system (Applied Biosystems) using SYBR Green PCR Master Mix according to the manufacturer’s recommendations. The equivalence of host DNA in the samples was confirmed by measurement of genomic IL-12p40 PCR product levels in the same samples. Purified \( T. cruzi \) DNA (American Type Culture Collection) was sequentially diluted for curve generation in aqueous solution containing equivalent amounts of DNA from uninfected mouse tissues. The following primers were used: \( T. cruzi \) minicircle specific-primers, GCTCTTGCCCACAMGGGTGC, where M = A or C (S35-forward) and CCAAGCAGGGATAGTTCCAG (S36-reverse); and genomic il-12p40, GTAGAGGTGGACTGACTCC (forward) and CAGATGGAAGGGCTCAGAG (reverse).

Histopathology

Spleens of LRG-47 KO and WT mice were harvested at 11 and 15 days of infection and fixed with buffered formalin. After paraffin embedding, sections were stained by H&E and examined at \( \times 20 \).

Statistics

The significance of differences between sample means was determined by Student’s \( t \) test. A value of \( p < 0.05 \) was considered significant.

Results

IFN-\( \gamma \)-dependent induction of LRG-47 by \( T. cruzi \)

To determine whether LRG-47 is induced during \( T. cruzi \) infection, real-time RT-PCR measurements of \( T. cruzi \) expression were performed in spleens of WT or IFN-\( \gamma \)-KO mice 7 days after i.p. infection with \( 10^7 \) \( Y \) strain blood-stage trypomastigotes. As shown in Fig. 1A, \( T. cruzi \) infection stimulated a >5-fold increase in LRG-47 expression in WT, but not in IFN-\( \gamma \)-deficient, animals. This finding confirmed that LRG-47 is induced by \( T. cruzi \) in vivo and that its expression is IFN-\( \gamma \)-dependent.

Mice deficient in LRG-47 display enhanced susceptibility to \( T. cruzi \) infection

To determine whether LRG-47 regulates host resistance to the parasite, LRG-47-deficient mice were infected with \( T. cruzi \), and survival and parasitemia were compared with those in WT and IFN-\( \gamma \)-deficient animals. LRG-47 KO mice displayed increased susceptibility to infection, exhibiting 100% mortality by day 19, whereas the WT control animals survived for >30 days (Fig. 1B). The median survival time of the infected LRG-47 KO mice (16 days) was comparable to that of IFN-\( \gamma \) KO animals (15 days). However, in contrast to the latter animals that displayed uncontrolled parasitemia beginning on day 8, peak parasitemia in LRG-47 KO mice occurred on days 10–11, followed by partial control of the infection for several days. This restriction of parasite growth was lost by day 14, as evidenced by a rebound in parasitemia (Fig. 1C) at the onset of death of the animals (Fig. 1B). The increased susceptibility of LRG-47 KO mice to \( T. cruzi \) infection was also apparent when parasite loads were measured in tissues (spleen, liver, and heart) by real-time PCR (Fig. 1D). Thus, although WT mice successfully limited parasite growth in these tissues by day 15, LRG-47 KO mice displayed increased parasite DNA levels during the same period, although they were lower than elevations seen in IFN-\( \gamma \) KO animals (Fig. 1D). Interestingly, tissue parasite loads were lower in the LRG-47 KO than in the WT mice when measured on day 8 of infection, an observation consistent with the delayed peak of parasitemia seen in the KO mice at the same time point (Fig. 1C).

LRG-47 KO mice display alterations in T cell activation, but unimpaired proinflammatory cytokine production, in response to \( T. cruzi \) infection

To determine whether T cells from infected LRG-47 KO mice are activated by the infection, we examined splenic T cell populations by flow cytometric analysis on days 8, 11, and 15 after parasite inoculation. \( T. cruzi \) infection induced activation of CD4+ and CD8+ T cells in the spleens of both WT and KO mice, with the percentages of these cells increasing during the course of the infection. Quantitative differences between the two types of animals were observed, with LRG-47 KO mice displaying somewhat reduced percentages of activated CD4+ T cells (CD62L(lo)CD44(high)) and increased numbers of activated CD8+ T cells (CD122(high) CD44(high)) compared with the infected WT controls (Fig. 2A). Similar trends were observed when the data were calculated in terms of absolute numbers of activated T cells per spleen (data not
FIGURE 2.  *T. cruzi*-infected LRG-47 KO mice display altered T cell activation, but increased proinflammatory cytokine responses. A, Splenic T cell activation was compared in infected WT and LRG-47 KO mice on days 8, 11, and 15 after infection by flow cytometric analysis. The numbers shown are the percentages of CD4+ (top panel) or CD8+ (lower panel) T cells. Each analysis is representative of three mice in each group. B, Expression of TNF, IFN-γ, IL-10, and NOS2 mRNAs were determined in spleens of infected WT (○) or LRG-47 KO (■) mice 7 days after infection. C, Serum IFN-γ and TNF levels were measured by ELISA on days 8, 11, and 15 after infection in infected WT (○) or LRG-47 KO (■) mice. The mean ± SE (n = 3) levels of each cytokine are shown. The data are representative of two separate experiments performed. *p < 0.05.

LRG-47 KO animals develop severe lymphopenia, anemia, and thrombocytopenia during *T. cruzi* infection

Because the increased susceptibility of LRG-47 KO animals to mycobacterial infection has been shown to be associated with reductions in lymphocytes and other blood elements, we asked whether similar changes occur in *T. cruzi*-infected LRG-47-deficient mice. This question was of particular relevance because *T. cruzi* infection is known to lead to hematological alterations in patients (22) as well as LRG-47-deficient experimental animals (16, 23, 24). On day 8 after infection, with the exception of mild lymphopenia, no major hematological defects were observed in either WT or KO mice (Fig. 3). However, at later time points, WT animals displayed mild anemia, lymphopenia, and thrombocytopenia and on day 15 exhibited lymphohemocytosis (Fig. 3) along with a dramatic increase in splenic cellularity (Fig. 4A). LRG-47 KO mice, in contrast, displayed more profound anemia, thrombocytopenia, and lymphopenia when examined at the same time points. The lymphopenia in the infected LRG-47 KO mice evident on day 15 was also apparent in a striking reduction in the size of the white pulp in spleen, which also showed evidence of decreased extramedullary hemopoiesis (arrows, Fig. 4B). These changes were accompanied by a dramatic loss in bone marrow cellularity in the LRG-47-deficient animals (Fig. 4A).

IFN-γ-treated, LRG-47-deficient macrophages display defective parasite killing despite unimpaired TNF and NOS2 expression

Macrophages represent an important site for *T. cruzi* replication and control in infected mice (25, 26), especially for reticulotropic strains such as the Y strain examined in this study. To determine whether LRG-47 regulates parasite growth in these cells, WT and KO BMMΦ were infected in vitro with *T. cruzi* (5:1 trypomastigotes/host cell) for 2 h, and the number of intracellular amastigotes per 200 macrophages was determined 1 and 48 h later. Although WT and LRG-47 KO macrophages were nearly equally susceptible to initial infection, the parasites survived and replicated better in the KO macrophages, as evidenced by an increased growth slope (θ = 558) vs the WT macrophages (θ = 250; Fig. 5A). Upon activation with IFN-γ, macrophages from both WT and KO mice significantly restricted parasite growth. However, although IFN-γ treatment resulted in a dose-dependent killing of parasites in WT macrophages (as demonstrated by negative θ in Fig. 5B), in KO macrophage the same cytokine doses resulted only in impaired parasite growth compared with untreated cells (Fig. 5B). Similar results were obtained when the same experiments were performed with peritoneal exudate macrophages (data not shown). To determine whether these differences observed in parasite control are due to a defect in either TNF or NOS2 induction, we measured mRNA levels for both mediators by real-time RT-PCR at 2 h after infection. As shown in Fig. 5C, the expression of both TNF and NOS2 was observed in IFN-γ-treated LRG-47 KO macrophages at levels that, in fact, exceeded those observed with similarly treated and infected WT macrophages.
LRGB-47 and NOS2 cooperate in the intracellular killing of T. cruzi by IFN-γ-activated macrophages

Previous studies have established a major role for NO as a mediator of parasite killing in T. cruzi-infected macrophages. The finding that NOS2 gene expression was not reduced (and was, in fact, increased) in IFN-γ/H9253-activated LRG-47 KO macrophages suggested that LRG-47 acts independently of NO induction. Therefore, we hypothesized that NOS2 and LRG-47 might act together as mediators of IFN-γ-induced intracellular control of parasite growth. To test this concept, WT and LRG-47 KO BMMφ were infected with...
T. cruzi and stimulated with different doses of IFN-γ in the presence or the absence of the NOS inhibitor l-NMMA. After 48 h, cultures were fixed and stained, and intracellular amastigotes were quantified. Macrophage from LRG-47-deficient mice were again found to be defective in their ability to restrict parasite growth, and this impairment was evident at each dose of IFN-γ treated (Fig. 6, top panel). l-NMMA-treated WT macrophages were able to partially restrict parasite replication (by ~30–40%) when stimulated with 200 U/ml IFN-γ (Fig. 6, bottom panel), but not with 20 or 2 U/ml. In contrast, when tested at this higher cytokine dose, LRG-47 KO macrophages treated with l-NMMA failed to show any detectable control of parasite growth. To further examine the interaction between LRG-47- and NOS2-dependent microbicidal mechanisms, lrg-47 expression was suppressed in macrophages by gripNA gene silencing (Fig. 7, A and B). As previously observed in LRG-47 KO macrophages (Figs. 5 and 6), parasite growth was significantly enhanced in lrg-47 gene-silenced WT macrophages (Fig. 7C). IFN-γ treatment caused a reduction in parasite number in both control and gene-silenced macrophages, although to a lesser degree in the latter. Importantly, no effect of IFN-γ addition on parasite proliferation was observed when LRG-47 expression was suppressed in NOS2-deficient macrophages (Fig. 7C), supporting an independent, but cooperative, function for these mediators in intracellular control of T. cruzi infection.

**Discussion**

In this study we investigated the role of the IFN-induced GTPase LRG-47 in host resistance to infection with T. cruzi, a protozoan pathogen known to be controlled by IFN-γ-dependent effector mechanisms. Previous reports indicated that LRG-47 is required for IFN-γ-mediated control of T. gondii (7, 27), Listeria monocytogenes (7), M. tuberculosis (10), and M. avium (11). In the murine T. cruzi infection model used in this study, we found that LRG-47 KO mice are nearly as susceptible as IFN-γ KO animals, in that they show comparable median survival times and display dramatically elevated tissue parasite burdens when examined on day 15. Nevertheless, T. cruzi-infected LRG-47 KO mice differed from IFN-γ KO animals in their blood parasite numbers displaying partial control during the first 2 wk of infection. This finding suggests that a distinct LRG-47-independent mechanism is able to limit systemic pathogen proliferation during the initial wave of parasitemia and that LRG-47 functions primarily at later times, exerting its effects on T. cruzi replication within tissues. Our additional experiments revealed two defects in T. cruzi-infected LRG-47 KO mice that may explain this loss in tissue control of parasite growth. The first defect is the development of profound and persistent lymphopenia, contrasting with the transient lymphocyte reduction seen in WT control animals; the second is an impairment in IFN-γ-mediated killing of the parasite by macrophages.

Previous studies in WT animals indicated that T. cruzi infection, after transient lymphopenia (16), induces a potent lymphoproliferative response evidenced by lymphocytosis associated with increased activation-induced cell death (28, 29). In contrast, infected LRG-47 KO animals were found to display a profound and permanent lymphopenia. As also documented in a previous study with M. avium infection, the observed lymphopenia is accompanied by thymic and splenic atrophy (11). This phenotype could be the result of a role of LRG-47 in negatively regulating the activation-induced cell death induced by T. cruzi infection. Nevertheless, lymphopenia is not the only hematological abnormality observed in infected LRG-47 KO mice. These animals also display enhanced and irreversible anemia and thrombocytopenia that was found to be associated with decreased infection-induced extramedullary hemopoiesis and general bone marrow failure. Thus, T. cruzi-infected LRG-47 KO mice exhibit a general defect in the production of multiple blood lineages, suggesting that LRG-47 may play an important role in regulating normal hemopoiesis during the host response to intracellular pathogens. Although the mechanism of this regulation is presently unclear, it is of interest that levels of IFN-γ and TNF were markedly elevated in LRG-47 KO mice at the time of cellular depletion. Changes in these cytokines have been linked to anemia, lymphopenia, and thrombocytopenia in previous studies of T. cruzi (16, 24) as well as viral infections (30). Moreover, similar changes have been observed in patients undergoing therapeutic interventions with either cytokine (31).

The macrophage is a primary niche for the replication of reticulotropic T. cruzi strains and thus serves as an important effector cell for parasite control. As demonstrated in this study, LRG-47, in addition to restricting parasite growth in macrophages, is required for effective IFN-γ-dependent killing of amastigotes, although some inhibition of pathogen replication is seen in its absence. It is
likely that this partial control is due to an NO-mediated mecha-
nism, because NOS2 activity was found to be unimpaired in LRG-
47-deficient macrophages, and treatment with L-NMMA com-
pletely inhibited the partial IFN-γ-dependent control of parasite
growth exhibited by LRG-47 KO macrophages. Therefore, these
observations suggest that although independent functions, LRG-47
and NO act together in mediating parasite control in activated
macrophages. A similar conclusion was drawn from studies of the role
of LRG-47 in the microbicidal activity of macrophages for M.
tuberculosis (10). As in that system, our findings are more con-
sistent with additive, rather than synergistic, roles of LRG-47 and
NO in intracellular pathogen control.

As described previously in the murine T. gondii model for an-
other IFN-induced GTPase, ITGPT, LRG-47- and NO-de-
dependent effector mechanisms may act preferentially at different
stages of T. cruzi infection. Previous studies have suggested that
NOS2 is critical during acute, but not chronic, infection with T.
cruzi, although this requirement appears to vary with the parasite
strain used. Thus, we and others have shown that NOS2 KO mice
become susceptible to Y or Colombiana strains during the acute phase of
T. cruzi infection (33–35) (data not shown). Moreover, T. cruzi-in-
fected animals can survive when NOS2 activity is inhibited during
chronic infection (34) or when infected NOS2 KO mice are given
short-term chemotherapy during early infection (36), suggesting a
parasitostatic role of NO in the acute, but not the chronic, phase of
Chagas disease. Nevertheless, NOS2 does not appear to be critical
for host resistance to less virulent strains of T. cruzi (37). We
speculate that an NOS2-mediated mechanism(s) accounts for the
early control of parasitemia seen in the Y strain-infected LRG-47
KO animals we studied. At present, the explanation for the appar-
et kinetic difference in NO and LRG-47 dependence in T. cruzi
infection is unclear, but it may relate to the different blood and
tissue sites invaded by the parasite during the progression of the
disease.

In their study on the effects of LRG-47 on macrophage control of
M. tuberculosis, MacMicking et al. (10) demonstrated a major
effect of the gene on IFN-γ-induced phagosome maturation and
lysosomal fusion. Because T. cruzi escapes into the cytosol soon
after host cell invasion, the same mechanism is not likely to ac-
count for the role of LRG-47 in macrophage killing of this proto-
zoan parasite. Nevertheless, during the first few hours of infection,
the organism resides transiently in phagosomes that undergo lyso-
somal fusion (15), a process that has been shown to be required for
successful host cell entry (14). Whether IFN-γ acts against the
parasite at this stage is unknown, but, if so, LRG-47 might function
in promoting T. cruzi killing by regulating some as yet to be iden-
tified factor involved in phagosome maturation or function.
LRG-47 has also recently been shown to promote macrophage
autophagy, a newly described mechanism for intracellular control
of bacterial infection (13). Nevertheless, whether autophagy plays
a role in IFN-dependent killing of protozoa has yet to be estab-
lished, and additional studies are therefore required to test a pos-
sible link between this mechanism and the influence of LRG-47 in
host resistance to T. cruzi.

Although the effects of LRG-47 on macrophage control of in-
tracellular pathogens and on infection-induced hemopoiesis have
been documented previously, the present study is the first to si-
multaneously document these mechanisms in the same animal
model. Undoubtedly, the existence of these dual defects in T.
cruzi-infected LRG-47 mice contributes to their marked suscepti-
ability. Nevertheless, it is not yet clear whether the observed mac-
rophage and hemopoietic dysfunctions observed in the KO animals
represent distinct independent effects of LRG-47 deficiency or are
functionally linked. For example, it is possible that the severe bone
marrow failure induced in these mice stems in part from the in-
creased parasite load resulting from the inability of activated mac-
rophages to control the infection. In contrast, the impaired para-
tosstatic function of LRG-47 macrophages may be the manifes-
tation of the same developmental defect that accounts for the
hemopoietic malfunction in the KO animals. Indeed, recent
experiments (C. Feng, unpublished observation) have demon-
strated the existence of a major functional defect in LRG-47-de-
dicient stem cells. Finally, although LRG-47 clearly influences
control of parasite proliferation, it is possible that the acute death
of these animals is due at least in part to the dramatic bone marrow
failure they experience.

The findings reported in this study support previous studies in-
dicating that IFN-γ signaling has pleiotropic effects on both im-
mune defense and homeostasis as well as hemopoietic function
(38–40). More importantly, they argue that LRG-47 is a critical
regulator of this shared pathway, and that the study of LRG-47
function may therefore shed important light on how IFN jointly
regulates these diverse immunological and hematological
parameters.

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Disclosures
The authors have no financial conflict of interest.

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CORRECTIONS


Figure 8 is incorrect. The corrected figure is shown below.


The second author’s middle initial was omitted. The correct name is Robert L. Ferris.


The eighth author’s last name was misspelled. The correct name is Leda Q. Vieira.

The ninth author’s last name was misspelled. The correct name is Hideaki Nakajima.

In Figure 2A, the three left hand dot plot panels from Ly9+/+ cells were mistakenly duplicated in the three right hand dot plot panels of Ly9−/− cells. The numbers in each of the quadrants are correct and the error does not change any interpretation in the article. The corrected figure is shown below.

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One of the first author’s affiliations was omitted. The corrected list of authors and affiliations is shown below.

Meng-Tsung Tien,2*†‡ Stephen E. Girardin,2*B éatrice Regnault,† Lionel Le Bourhis,§ Marie-Agnès Dillies,† Jean-Yves Coppée,† Raphaëlle Bourdet-Sicard,§ Philippe J. Sansonetti,3* and Thierry Pédrón*

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In Figure 1B, the WT Ca flux data line is missing from the Ca flux graph. The corrected figure is shown below.

In Discussion, the last reference in the paper is incorrect. The corrected sentence and reference are shown below.

It is known that the cytoplasmic domains of several components of the TCR complex tend to homo-oligomerize at high concentrations (41); perhaps ligand-induced clustering of the TCR drives the cytoplasmic domains of proximal receptors to rearrange, exposing the Nck binding epitope and propelling other signaling cascade processes.


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In Discussion, in the second sentence of paragraph six, 10S-HDNA should have been 10S-HDHA. The corrected sentence is shown below.

Recently, classic steric analysis of 10S-HDHA and the formation of 10,20-diHDHA and 17-H(p)DHA were reportedly optimized for the plant LOs (49).

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The title of the article is incorrect. The corrected title is shown below. The error has been corrected in the online version, which now differs from the print version as originally published.

Thymocyte Negative Selection Is Mediated by Protein Kinase C- and Ca2+-Dependent Transcriptional Induction of Bim