MAJOR ARTICLE

Turnover of Neutrophils Mediated by Fas Ligand Drives *Leishmania major* Infection

Flávia L. Ribeiro-Gomes,[1] Maria Carolina A. Moniz-de-Souza,[1] Valeria M. Borges,[3] Marise P. Nunes,[2] Marcio Mantuano-Barradas,[1] Heloisa D'Ávila,[2] Patricia T. Bozza,[2] Vera L. Calich,[4] and George A. DosReis[1]

[1]Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro, and [2]Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, [3]Centro de Pesquisas Gonçalo Moniz, Fiocruz, Salvador, and [4]Instituto de Ciências Biomédicas, University of São Paulo, São Paulo, Brazil

Apoptosis mediated by Fas ligand (FasL) initiates inflammation characterized by neutrophilic infiltration. Neutrophils undergo apoptosis and are ingested by macrophages. Clearance of dead neutrophils leads to prostaglandin- and transforming growth factor–β–dependent replication of *Leishmania major* in macrophages from susceptible mice. How *L. major* induces neutrophil turnover in a physiological setting is unknown. We show that BALB/c FasL-sufficient mice are more susceptible to *L. major* infection than are FasL-deficient mice. FasL promotes the apoptosis of infected resident macrophages and attracts neutrophils. Furthermore, FasL-sufficient neutrophils exacerbate *L. major* replication in macrophages, whereas FasL-deficient neutrophils induce parasite killing. These contrasting effects are due to delaying apoptosis and the clearance of FasL-deficient neutrophils. The transfer of neutrophils exacerbates infection in FasL-sufficient mice but reduces infection in FasL-deficient mice. Depletion of neutrophils abolishes the susceptibility of FasL-sufficient mice. These data illustrate a deleterious role of the FasL-mediated turnover of neutrophils on *L. major* infection.

Neutrophils play a nonphagocytic immunoregulatory role in parasitic infections [1]. Indeed, neutrophils secrete reactive oxidants, cytokines, chemokines, and proteases that act on immune and inflammatory cells. However, the life span of neutrophils is tightly controlled by phagocytic clearance, and extended neutrophil survival leads to inflammation [2]. Neutrophils undergo constitutive apoptosis and are ingested by macrophages [3]. The ingestion of apoptotic neutrophils inactivates macrophages through the secretion of prostaglandin and transforming growth factor (TGF)–β [4]. Furthermore, the phagocytic clearance of dead cells drives the TGF-β–dependent replication of protozoal parasites in macrophages [5, 6]. Therefore, the turnover of inflammatory neutrophils could drive infection by intracellular pathogens that use macrophages as host cells. Neutrophils play a deleterious role in infection with *Leishmania major* [6, 7]. The clearance of dead neutrophils leads to prostaglandin- and TGF-β–dependent replication of *L. major* in macrophages [6]. However, how *L. major* induces the turnover of neutrophils in a physiological setting is unknown. Fas ligand (FasL) is a membrane-bound and shed surface protein that belongs to the tumor necrosis factor gene family and is the natural counterreceptor for the Fas death receptor [8]. Apoptosis initiated by FasL leads to inflammation mediated by neutrophils [9–12]. We show that BALB/c FasL-sufficient mice are more susceptible to *L. major* infection than are FasL-deficient mice. Infection induces FasL-dependent resident macrophage apoptosis and attracts neutrophils. Compared with FasL-sufficient neutrophils, delayed apoptosis and clearance of FasL-deficient neutrophils leads to enhanced macrophage control of parasite replication. The present data show a deleterious role of the FasL-mediated turnover of neutrophils on *L. major* infection.

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Reprints or correspondence: Dr. George A. DosReis, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Bloco G, Ilha do Fundão, Rio de Janeiro RJ 21944-970, Brazil (gdosreis@biof.ufrj.br).

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MATERIALS AND METHODS

Mice, parasites, and infection. BALB/c mice—either wild-type *(wt)* or FasL-deficient *gld* mutant mice [11]—were from the Oswaldo Cruz Institute animal care facility. Mice were infected subcutaneously with 2×10^6 *L. major* strain LV39 (MRHO/Sv/59/P) promastigotes. Disease was scored by footpad swelling as measured by a micrometric caliper and by parasite burden in lymph-node cells, as described elsewhere [6]. Briefly, after 41 days of infection, draining lymph nodes of individual mice were collected, and lymph-node cells were suspended at 1×10^7 viable cells/mL in serum-free Dulbecco's modified Eagle medium (DMEM). Cells were serially diluted in triplicate in supplemented Schneider medium [13] and were incubated at 26°C. Parasite load was measured by counting viable promastigotes after 7 days in culture. For the measurement of rapid cellular responses to infection, mice were injected intraperitoneally (ip) with saline or with 1×10^6 promastigotes. Twenty hours later, peritoneal exudate cells were collected. Aliquots were stained by Giemsa to determine differential leukocyte counts. Cells were treated with FcBlock (BD Biosciences), stained with fluorescein isothiocyanate–labeled antibody specific for F4/80 (Caltag) and phycoerythrin-labeled antibodies specific for Mac-1 or FasL (BD Biosciences), and analyzed by flow cytometry (FCM). Cells were also adhered to plastic wells, and adherent macrophages were transferred to supplemented Schneider medium [13]. After 5 days, the intracellular load of parasites was determined as described elsewhere [14]. Parasite loads from different mice were normalized for a fixed number of 10^6 macrophages, after individual proportions of macrophages were measured in stained aliquots of peritoneal exudate cells. All experiments were approved by an institutional committee for the humane care and use of animals.

Macrophage apoptosis and chemokine secretion. Peritoneal macrophages were infected in vitro with *L. major* promastigotes at a ratio of 1:10 for 20 h in the presence of IgG or a neutralizing antibody specific for FasL (5 μg/mL, clone MFL3; BD Biosciences) or in the presence of Fas/Fc or control CD30/Fc chimeric proteins (0.5 μg/mL; R&D Systems). Culture medium consisted of DMEM supplemented with 10% fetal calf serum (FCS). To determine the number of adhered macrophages, cultures done under glass coverslips were washed and stained with Giemsa. To analyze DNA fragmentation and chemokine secretion, cultures were done on plastic. Supernatants were assayed for fragmented DNA on agarose gels as described elsewhere [15] and for chemokines by sandwich ELISAs according to the manufacturers' instructions (BD for macrophage inflammatory protein [MIP]–1α and R&D Systems for KC).

Neutrophils, inflammatory macrophages, and infection. Neutrophils were collected 7 h after the ip injection of thioglycollate broth into noninfected mice (Difco). Neutrophils were depleted of adherent cells [16] and further purified by Percoll density-gradient centrifugation [17], yielding cells that were >95% Gr-1 positive by FCM. Neutrophils were killed by heating for 30 min at 56°C, as described elsewhere [18], yielding cells with characteristics of late apoptotic cells—that is, condensed chromatin but permeable to trypan blue. Inflammatory macrophages were collected 4 days after the ip injection of thioglycollate into noninfected mice. Exudate cells were infected with *L. major* promastigotes (1:10) in DMEM–10% FCS. After 4 h, extracellular parasites and nonadherent cells were removed. Macrophages were incubated with live or killed neutrophils in 48-well vessels that contained 0.5 mL of DMEM supplemented with 1% Nutridoma-SP (Boehringer-Mannheim), instead of FCS. Hamster IgG, an antibody specific for FasL (5 μg/mL), TGF-β receptor II/Fc chimeric protein, thrombopoietin receptor/Fc chimeric protein (0.5 μg/mL; both from R&D Systems), deferoxamine (100 mmol/L; Sigma), and L-N6-(1-iminoethyl)-lysine (L-NIL; 1 mmol/L; Sigma) were added. After 3 days, cultures were washed, macrophages were transferred to Schneider medium, and the intracellular load of parasites was measured as described elsewhere [6, 14]. In some experiments, the levels of nitrite production were measured by the Griess reaction [5] in culture supernatants obtained after 2 days in culture (5×10^5 macrophages plus 5×10^6 neutrophils were cultured).

Transfer and depletion of neutrophils. Mice were infected with *L. major* in both footpads and received 4×10^6 syngeneic neutrophils in the right footpad. After 10 days, parasite loads were determined in left and right draining lymph nodes [6]. To deplete neutrophils, mice received 5 ip injections of 100 μg of IgG or an antibody specific for Gr-1 [19], given at days 1, 2, 5, 8, and 11 after *L. major* infection. After 13 days, parasite loads were determined in draining lymph nodes [6].

Statistical analysis. The in vitro cultures were done in triplicate, and each experiment was repeated at least twice. Data represent the mean ± SE of representative experiments. For in vivo experiments, data show means and values from individual mice. Data derived from neutrophil recruitment and ex vivo parasite loads of macrophages were normalized by square-root transformation before statistical analysis. Data were analyzed by Student's *t* test for independent samples by use of SigmaPlot for Windows (version 4.0; SPSS). Differences with $P \leq .05$ were considered to be significant.

RESULTS

Responses of FasL-deficient mice to* L. major *infection. We asked whether FasL regulates infection by *L. major.* We compared FasL-sufficient *(wt)* and FasL-deficient *(gld)* BALB/c mice for susceptibility to *L. major* infection. We found that *gld* mice had significant reductions in footpad lesion size at 9, 16, and 34 days after infection (figure 1*A*–1*C*). After 41 days, the parasite load in lymph nodes from FasL-sufficient mice was 4–5-

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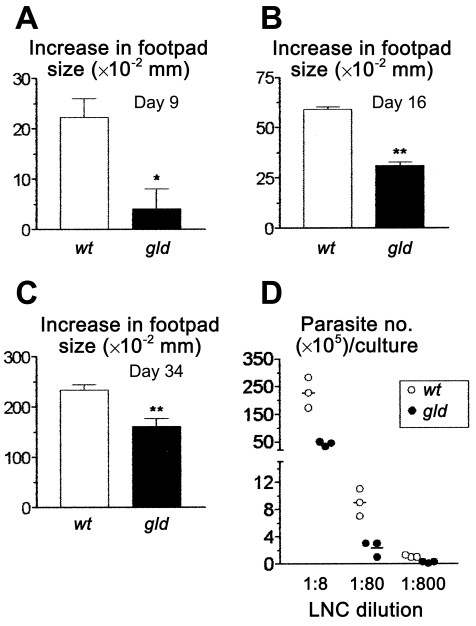

Figure 1. Fas ligand expression and increased susceptibility to *Leishmania major* infection. *A–C,* Footpad increase in wild-type *(wt)* and *gld* mice (4 animals each) after the indicated days of infection (day 9, $P < .05$; days 16 and 34, $P < .01$). *D,* Parasite loads in draining lymph-node cells (LNC) after 41 days of infection ($P < .01$). Each point represents an individual mouse.

fold higher than that in lymph nodes from FasL-deficient mice (figure 1*D*).

We investigated whether *L. major* infection induces the FasL-dependent death of resident macrophages. Indeed, the ip injection of *L. major* promastigotes into *wt* mice markedly reduced the number of peritoneal Mac-1^{hi} macrophages, as measured by FCM (see figure 2*A* and 2*B* for a representative experiment: 22.5% ± 2.7% Mac-1^{hi} F4/80^{+} cells after PBS and 8.5% ± 1.9% cells after *L. major,* 3 mice/group). Injection into FasL-deficient mice did not induce any loss of Mac-1^{hi} macrophages (see figure 2*C* and 2*D* for a representative experiment: 20.1% ± 0.6% Mac-1^{hi} F4/80^{+} cells after PBS and 19.3% ± 3.4% cells after *L. major,* 3 mice/group). Injection of *L. major* up-regulated FasL expression in the remaining Mac-1^{hi} macrophages from *wt* mice (figure 2*E*), whereas Mac-1^{lo} and Mac-1–negative cells did not up-regulate FasL expression. We investigated the death of resident peritoneal macrophages in vitro. Culture with *L. major* in the presence of control hamster IgG induced a 50% cell loss in adherent macrophages from *wt* mice after 20 h (figure 3*A*, left). Macrophage cell loss was completely prevented by a neutralizing antibody specific for FasL (figure 3*A*, left), and *L. major* did not induce cell loss in macrophages from *gld* mice (figure 3*A*, right). Analysis of DNA fragmentation in supernatants derived from macrophage monolayers confirmed that the apoptosis of macrophages was induced by *L. major* and that its complete blockade was induced by treatment with a Fas/Fc chimeric protein (figure 3*B*). Culture of resident macrophages from *wt* mice with *L. major* for 8 h increased secretion of the chemokine KC, and KC secretion could be blocked by Fas/Fc but not by a control chimeric protein (figure 3*C*). In contrast, culture of macrophages from *gld* mice with *L. major* did not increase KC secretion above background levels (data not shown). Furthermore, treatment of *wt* macrophages with *L. major* for 20 h induced the secretion of the chemokine MIP-1α (figure 3*D*), and MIP-1α secretion could be blocked with a neutralizing antibody specific for FasL (figure 3*D*). Injection of *L. major* promastigotes induced the ip extravasation of neutrophils in *wt* mice (figure 3*E*). Background ip levels of neutrophils were higher in *gld* mice (figure 3*E*). However, *L. major* did not increase numbers of neutrophils in *gld* mice (figure 3*E*). We also investigated the intracellular parasite load of peritoneal macrophages recov-

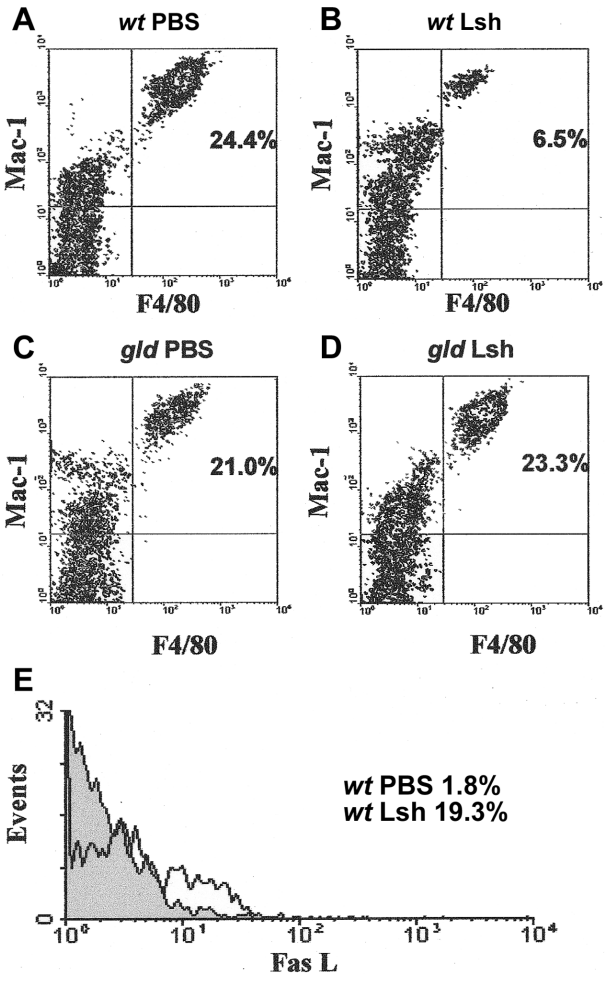

Figure 2. *Leishmania major* (Lsh)–induced demise of Mac-1^{hi} macrophages in Fas-ligand (FasL)–sufficient mice. *A* and *B,* Percentages of Mac-1^{hi} macrophages after an intraperitoneal (ip) injection of saline PBS or Lsh in wild-type *(wt)* mice. *C* and *D,* Percentages as in panels A and B for *gld* mice. *E,* Percentages of FasL-positive cells in Mac-1^{hi} macrophages from *wt* mice injected with saline PBS or Lsh. Experiment representative of 3 experiments with similar results.

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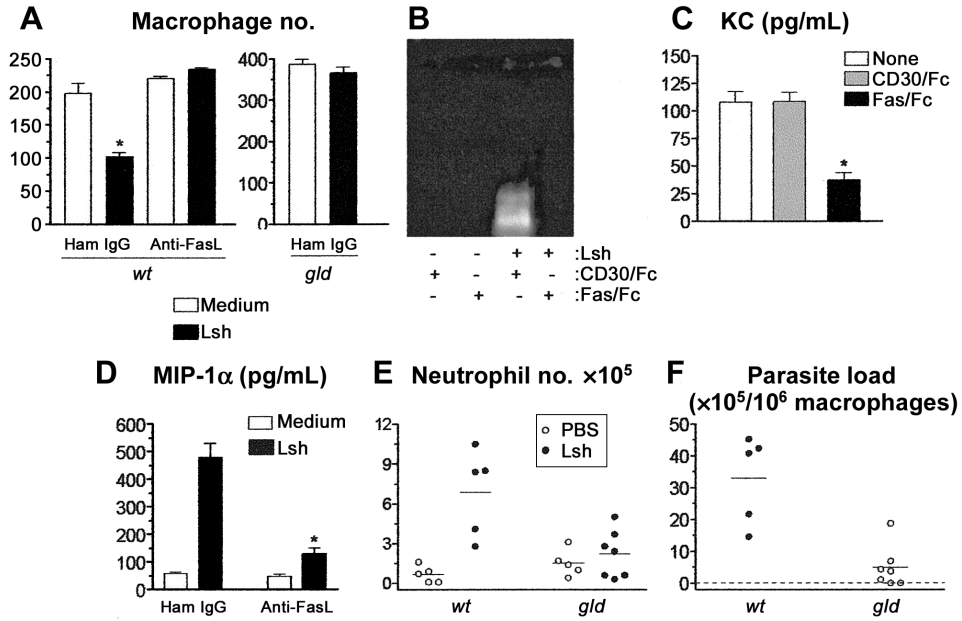

Figure 3. *Leishmania major* (Lsh) induction of resident macrophage apoptosis and neutrophil extravasation in Fas-ligand (FasL)–sufficient mice. *A, L. major* promastigotes induction of cell loss in macrophages from wild-type *(wt)* mice in vitro (*$P < .01$) in the presence of hamster IgG (ham IgG) but not in the presence of antibody specific for FasL (anti-FasL). Right, No induction by Lsh of cell loss in macrophages from *gld* mice. *B,* Prevention of DNA fragmentation in supernatants from macrophages treated with Lsh by soluble Fas/Fc but not by a control CD30/Fc construct. *C,* Blocked secretion of KC elicited by Lsh in macrophages *(white bar)* by Fas/Fc (*black bar;* *$P < .05$) but not by a control CD30/Fc construct *(gray bar). D,* Prevention of secretion of macrophage inflammatory protein (MIP)–1α induced by Lsh in macrophages an antibody specific for anti-FasL (*$P < .01$). *E,* Neutrophil extravasation in *wt* ($P < .05$) but not in *gld* mice. *F,* Higher parasite load in macrophages infected in vivo with *L. major* in *wt* vs. *gld* mice ($P < .01$). Data in *A–D* are representative of at least 2 independent experiments with similar results. Data in *E* and *F* are mean and values from individual mice.

ered 20 h after in vivo infection. The mean parasite load of recovered macrophages from *wt* mice was >6-fold higher than that of macrophages from *gld* mice (figure 3*F*). Because a 20-h period is not sufficient for intracellular differentiation to amastigotes and amastigote replication, these data suggest that macrophages from *wt* mice were more permissive of parasite invasion than were macrophages from *gld* mice.

Requirement of FasL for rapid neutrophil apoptosis and increased parasite replication. We investigated how infected macrophages control *L. major* replication after interaction with highly purified neutrophils from *wt* and *gld* mice. Inflammatory macrophages were resistant to apoptosis in the presence of parasites or neutrophils (data not shown). Inflammatory macrophages infected with *L. major* amastigotes recognized and ingested dead neutrophils (figure 4*A*). In agreement with previous results [6], both live and dead neutrophils from *wt* mice exacerbated *L. major* replication in macrophages from *wt* mice (figure 4*B*). However, live neutrophils from *gld* mice reduced *L. major* replication in *gld* macrophages (figure 4*B*). Dead neutrophils from *gld* mice exacerbated parasite replication in a manner similar to neutrophils from *wt* mice (figure 4*B*). Cell-mixing experiments demonstrated that live neutrophils, and not macrophages, regulate opposite outcomes of *L. major* infection (figure 4*B*).

We investigated the role of FasL in the spontaneous death of neutrophils from *wt* mice. In the presence of control IgG, highly purified inflammatory neutrophils rapidly died in vitro, with a $t_{1/2}$ of 4.8 h (figure 4*C,* left). After 24 h, no viable cells were left. In the presence of a neutralizing antibody specific for FasL, neutrophils still underwent spontaneous death. However, anti-FasL antibody induced a significant delay in spontaneous apoptosis that had a $t_{1/2}$ of 8.0 h (figure 4*C,* middle). Furthermore, 15% of neutrophils remained viable after 24 h. In agreement with these results, neutrophils from *gld* mice underwent delayed spontaneous death, compared with that in neutrophils from *wt* mice (figure 4*C,* right). Therefore, FasL significantly accelerates the rate of neutrophil apoptosis. We investigated whether the shortening of the neutrophil life span as mediated by FasL was responsible for the increased replication of *L. major* in macrophages. Indeed, an antibody specific for FasL completely prevented the exacerbation of macrophage parasite growth that had been induced by live neutrophils (figure 4*D*). Anti-FasL antibody had no effect on inflammatory macrophages alone or on the deleterious effect promoted by

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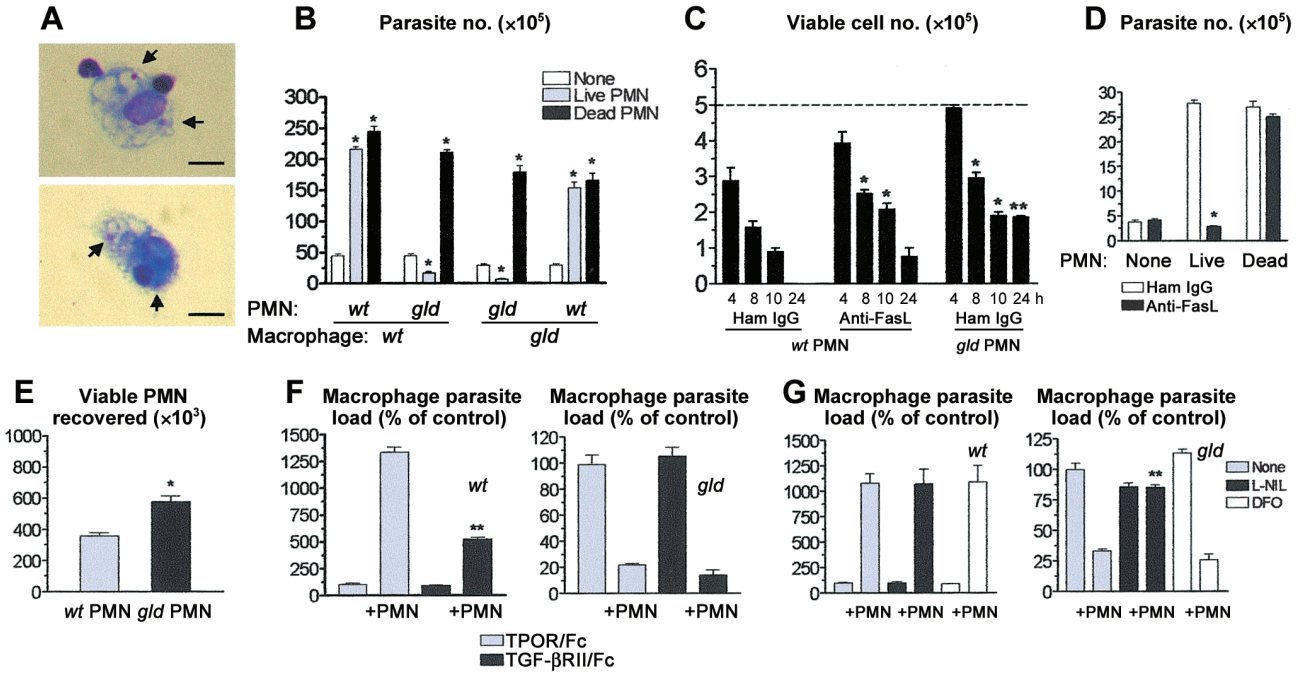

Figure 4. Promotion of *Leishmania major* replication in macrophages by neutrophils (polymorphonuclear cells [PMNs]) expressing Fas ligand (FasL). *A, Top,* Adhesion and phagocytosis of 2 dead neutrophils by a wild-type *(wt)* macrophage infected with 2 *L. major* amastigotes *(arrows). Bottom,* phagocytosis of a neutrophil by a macrophage infected with 2 amastigotes *(arrows).* Note intense vacuole formation in macrophages (scale bar, 5 μm). *B,* Exacerbation of *L. major* growth by neutrophils expressing FasL *(wt)* in *wt* and *gld* macrophages. Neutrophils deficient in FasL *(gld)* induce *L. major* killing. Dead neutrophils exacerbate *L. major* growth irrespective of genotype (all results, $^*P < .01$). *C,* Acceleration of neutrophil death by FasL. Antibody specific for FasL (anti-FasL; middle), but not hamster IgG (ham IgG; left), extended the life span of neutrophils from *wt* mice ($^*P < .05$). Neutrophils lacking FasL (*gld* PMN; right) had an extended life span, compared with that of neutrophils from *wt* mice ($^*P < .05$; $^{**}P < .01$). The dashed line indicates viable cell input. *D,* Blockade of FasL prevention of *L. major* growth promoted by live neutrophils ($^*P < .01$) but not growth promoted by dead neutrophils. *E,* Delayed clearance of neutrophils lacking FasL (*gld* PMN), compared with that of neutrophils from *wt* mice ($^*P < .01$), when either are incubated with *L. major*–infected macrophages from *wt* mice. *F,* Reduction in parasite replication induced by live neutrophils from *wt* mice by neutralizing transforming growth factor (TGF)–β receptor II/Fc chimeric protein (left; $^{**}P < .01$ vs. thrombopoietin receptor/Fc chimeric protein [TPOR/Fc]) but no affect on parasite killing induced by live neutrophils from *gld* mice *(right).* All effects induced by PMNs were significant ($P < .01$). *G,* Parasite killing induced by PMN from *gld* mice dependent on NO production. Scavenger of reactive oxygen species (deferoxamine [DFO]) and the inhibitor of NO production L-N6-(1-iminoethyl)-lysine (L-NIL) did not affect parasite growth induced by live PMNs in macrophages from *wt* mice *(left).* L-NIL, but not DFO, blocked parasite killing induced by PMNs from *gld* mice (*right,* $^{**}P$ = NS in the presence of L-NIL). All other effects of PMNs were significant ($P < .01$). Data presented in *B–G* are experiments representative of at least 2 experiments with identical results.

dead neutrophils (figure 4*D*). Next, we compared the rate of clearance of live neutrophils from *wt* and *gld* mice by infected macrophages from *wt* mice (figure 4*E*). In the presence of infected macrophages, the spontaneous death of neutrophils was delayed, and viable cells were recovered after 20 h in culture. However, the recovery of neutrophils from *gld* mice was significantly higher than that of neutrophils from *wt* mice (figure 4*E*), which indicates that FasL accelerates the clearance of neutrophils by macrophages.

Macrophage interactions with neutrophils from *wt* and *gld* mice were mechanistically and molecularly distinct. Parasite growth induced by neutrophils from *wt* mice required cell contact with macrophages, but parasite killing induced by live neutrophils from *gld* mice did not. Furthermore, parasite growth induced by neutrophils from *wt* mice could be blocked with a soluble TGF-β receptor chimeric construct (figure 4*F*, left). Parasite killing induced by neutrophils from *gld* mice did not involve TGF-β (figure 4*F*, right). Parasite growth driven by neutrophils from *wt* mice was independent of the production of both NO and reactive oxygen species (figure 4*G*, left). Parasite killing induced by neutrophils from *gld* mice was independent of oxygen species but dependent on NO production, given that killing was prevented by the addition of the NO synthase inhibitor L-NIL (figure 4*G*, right). To investigate whether neutrophils from *gld* mice induce increased NO secretion, macrophages from *wt* mice were cultured with neutrophils from either *wt* or *gld* mice, and the levels of nitrites in culture were measured by the Griess reaction. The results of 1 experiment, which were reproduced in a repeat experiment, confirmed that neutrophils from *gld* mice induce increased NO production by

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macrophage/neutrophil cultures (macrophages alone from *wt* mice, 2.22 ± 0.12 μmol/L nitrites; macrophages plus neutrophils from *wt* mice, 6.95 ± 0.12 μmol/L nitrites; macrophages plus neutrophils from *gld* mice, 23.01 ± 4.49 μmol/L nitrites [$P < .05$ for neutrophils from *wt* vs. *gld* mice]; neutrophils alone from *wt* mice, 1.40 ± 0.20 μmol/L nitrites; and neutrophils alone from *gld* mice, 2.22 ± 0.08 μmol/L nitrites). These data suggest that extended neutrophil survival controls parasite replication through the release of soluble mediators that promote NO production in macrophages.

In vivo infection driven by FasL expression by neutrophils. We investigated the role of FasL in the function of neutrophils in vivo. We transferred inflammatory neutrophils to the right footpad of *wt* and *gld* mice that had been injected with *L. major* in both footpads. The transfer of neutrophils from *wt* mice markedly exacerbated *L. major* infection in *wt* recipients (figure 5*A*). However, the transfer of neutrophils from *gld* mice reduced the parasite burden in *gld* recipients (figure 5*B*). We also depleted *wt* and *gld* mice of neutrophils. In mice treated with control IgG, parasite loads were 4-fold higher in *wt* mice, compared with those in *gld* mice (figure 5*C*). Neutrophil depletion markedly reduced parasite loads in *wt* mice (8-fold) but marginally increased parasite loads in *gld* mice (figure 5*C*). As a result, after neutrophil depletion, parasite loads were 5-fold lower in *wt* mice, compared with those in *gld* mice (figure 5*C*). Therefore, neutrophils are responsible for the divergent phenotypes of *wt* and *gld* mice. Only neutrophils expressing FasL are deleterious to the host.

DISCUSSION

Our results have demonstrated that the turnover of neutrophils as mediated by FasL is exploited by *L. major* to drive infection in susceptible hosts. The phagocytic removal of apoptotic neutrophils inactivates macrophages through the secretion of prostaglandin and TGF-β [4]. Furthermore, interactions with dead neutrophils lead to prostaglandin- and TGF-β–dependent replication of *L. major* in macrophages [6]. Therefore, the turnover of inflammatory neutrophils could drive infection by *L. major.* However, how *L. major* induces neutrophil turnover in a physiological setting is unknown. Because the apoptosis induced by FasL attracts neutrophils [9–12], we investigated the role of FasL in infected, susceptible mice.

Our results have indicated that the expression of FasL increases susceptibility to *L. major* infection at 2 distinct steps of neutrophil turnover. First, *L. major* induces the FasL-dependent apoptosis of Mac-1^{hi} resident macrophages, concomitant with chemokine secretion and neutrophil extravasation. A previous study showed similar results after the injection of vesicles that contained FasL into healthy mice [12]. Our data have demonstrated that the increased secretion of KC and MIP-1α promoted by *L. major* is dependent on FasL. However, previous studies have demonstrated that FasL engagement in macrophages leads to the secretion of additional cytokines and chemokines, such as interleukin-1β, MIP-2, and MIP-1β [12]. The chemokines important for neutrophil recruitment in our model were not determined. Furthermore, resident macrophages exposed to *L. major,* but not control macrophages, expressed FasL. Further experiments are necessary to characterize the mechanism of FasL expression that is induced by *L. major.*

Second, our results have demonstrated that FasL accelerates the rate of constitutive neutrophil death and that this accelerated death is essential for *L. major* replication within macrophages. Previous studies have implicated FasL in neutrophil apoptosis, either directly [20] or indirectly through the secretion of soluble FasL by macrophages [21]. Our results agree with an autocrine role of FasL in neutrophil apoptosis [20]. The tissue life span determines the inflammatory properties of neutrophils [2]. Delaying apoptosis prolongs the functional longevity of neutrophils [22], leading to neutrophil accumulation and inflammation [23, 24]. However, the onset of apoptosis down-regulates

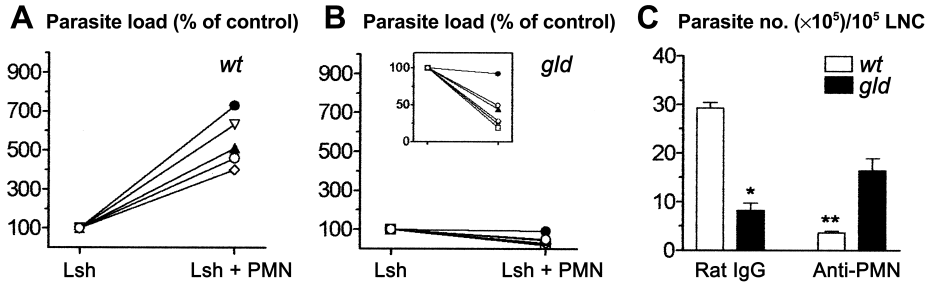

Figure 5. Neutrophils (polymorphonuclear cells [PMNs]) expressing Fas ligand (FasL) and exacerbation of *Leishmania major* (Lsh) infection. *A* and *B,* Local transfer of syngeneic neutrophils and exacerbation of infection by *L. major* in draining lymph-node cells (LNC) of wild-type *(wt)* mice (*A; P* < .01), but reduction of infection in *gld* mice (*B; P* < .01). Data are from individual mice and were amplified for *gld* mice *(inset). C,* Greater susceptibility of FasL-sufficient *(wt)* mice to *L. major* infection than that of FasL-deficient *(gld)* mice (parasite loads, **P* < .05); and increased susceptibility is abolished by treatment with a depleting antibody specific for neutrophils (anti-PMN) but not by control IgG (rat IgG). ***P* < .01.

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the proinflammatory activities of neutrophils [25, 26]. Contrary to neutrophils from *wt* mice, live neutrophils from *gld* mice underwent delayed apoptosis, which resulted in the delayed clearance of neutrophils and increased macrophage control over parasite replication. In agreement with this, neutralizing anti-FasL antibody delayed neutrophil death in *wt* mice and markedly reduced parasite replication in macrophages.

In agreement with the in vitro results, granulocyte depletion by treatment with anti–Gr-1 antibody abolished the increased susceptibility of *wt* mice to infection, which suggests a deleterious role of neutrophils in vivo. Although neutrophil depletion with anti–Gr-1 has been used in several studies, we cannot disregard the involvement of other myeloid cell populations that express the Gr-1 antigen [27, 28]. Parasite loads in *gld* mice treated with anti–Gr-1 were higher than those in *wt* mice. One possibility to explain this finding would be that, in the absence of neutrophils, functional abnormalities of T lymphocytes in *gld* mice [29] are responsible for the deficient control of *L. major* infection.

Recently, an alternative deleterious effect of neutrophils has been described in human cells [30]. Neutrophils infected with *L. major* undergo apoptosis and are ingested by macrophages, which leads to the transfer of parasites to macrophages [30]. In the present study, we did not investigate this mechanism. Instead, we used uninfected neutrophils cultured with infected macrophages. It is likely that both mechanisms coexist at the site of infection. However, the efficiency of parasite transfer from dying neutrophils remains to be investigated in mice.

In contrast to our results, studies in mice genetically resistant to *L. major* have indicated a protective role of FasL [31, 32]. Recently, we [6] and others [33] have demonstrated distinct populations of type 1 and type 2 neutrophils that direct macrophage activation to microbicidal or alternatively activated states, respectively. Contrary to that of neutrophils in BALB/c mice, the clearance of neutrophils in B6 mice is proinflammatory and leishmanicidal [6]. These findings could explain a protective role of FasL in resistant strains [31, 32]. Our data indicate immunoregulatory roles of FasL in parasite infection that include the promotion of resident macrophage apoptosis, neutrophil recruitment, and rapid phagocytic removal of senescent neutrophils. Apoptosis of resident macrophages as mediated by FasL could represent a general signal that alerts the immune system of the presence of an invading pathogen. These findings could be helpful in the design of new therapies for *L. major* infection in genetically susceptible individuals.

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