A role for IgG immune complexes during infection with the intracellular pathogen *Leishmania*

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We examined the role of immunoglobulin (Ig)G antibodies in mediating host defense to the intracellular parasite, Leishmania. We show that IgG not only fails to provide protection against this intracellular pathogen, but it actually contributes to disease progression. The J_H strain of BALB/c mice, which lack IgG because they have a targeted deletion in the Ig heavy chain (J) locus, were more resistant to infection with *Leishmania major* than were normal BALB/c mice. However, the passive administration of anti-Leishmania IgG caused J_H mice to develop large lesions containing high numbers of parasites. Antibody administration correlated with an increase in interleukin (IL) 10 production in lesions, and blocking the murine IL-10 receptor prevented antibody-mediated disease exacerbation. In human patients with active visceral leishmaniasis, high IgG levels are predictive of disease. Patients with ongoing disease had high IgG antibody titers and no delayed-type hypersensitivity (DTH) responses to Leishmania antigens. This pattern was reversed upon disease resolution after treatment, resulting in a decrease in total IgG, which was accompanied by a progressive increase in DTH responsiveness. We conclude that IgG can cause a novel form of immune enhancement due to its ability to induce IL-10 production from macrophages.

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Abbreviations used: αLm, α–*L. major*; ADE, antibody-dependent immune enhancement; DTH, delayed-type hypersensitivity; LMW-HA, low molecular weight hyaluronic acid; VL, visceral leishmaniasis.

Leishmania are protozoan parasites that reside predominantly, if not exclusively, in host tissue macrophages. This organism is a significant human pathogen, causing a spectrum of diseases in man (for reviews see references 1, 2). The disease is transmitted by infected phlebotamine sandflies, which transfer the flagellated promastigote form to the host. These organisms rapidly gain entry into phagocytic leukocytes, and transform into the oval, nonmotile amastigote form. Amastigotes replicate intracellularly within macrophage phagolysosomes, and spread the infection to adjacent macrophages.

The murine model of cutaneous leishmaniasis caused by *Leishmania major* has served as a paradigmatic model in which to study cellular immunity to an intracellular pathogen. In this model, the development of a Th1 response by resistant strains of mice led to the production of IFN-γ and the development of small lesions with relatively few parasites. However, in the BALB/c mouse, the inappropriate induction of a Th2 response led to the development of larger lesions with high parasitemia. These mice fail to control the disease and eventually succumb to infection. This model has done much to help us understand the events leading to T cell biasing and immune deviation.

Recently, IL-10 has been identified as an important mediator of susceptibility in both murine cutaneous and visceral leishmaniasis (VL; references 3–5). This cytokine has long been shown to be elevated in humans suffering with VL (6). In a recent paper, Murray et al. demonstrated that macrophage-derived IL-10 can increase susceptibility to *L. major* (7). In the present work, we examine a potential mechanism whereby IL-10 is produced by macrophages during Leishmania infection.

Classically activated macrophages play a central role in cell-mediated immunity. These cells produce an array of proinflammatory cytokines, which have the potential to contribute to autoimmune pathologies when overproduced (8). We have previously shown that macrophages activated in the presence of immune complexes shut off IL-12 biosynthesis (9) and secrete high levels of IL-10 (10).

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The prediction from these previous in vitro observations was that immune complexes could adversely influence the development of cell-mediated immunity by virtue of this reciprocal alteration in cytokine production (11). In the present studies, we examined the role of IgG during infections caused by the intracellular protozoan parasite *Leishmania* spp. We show that in both experimental animals infected with *L. major*, and in humans with VL, the presence of IgG immune complexes correlates with an inability to resolve infections. Thus, these studies demonstrate that IgG immune complexes can be detrimental to a host infected with this intracellular pathogen.

RESULTS

IgG promotes lesion progression in mice lacking IgG

To determine a role for IgG antibodies in the host immune response to *Leishmania* spp., we infected normal BALB/c mice with *L. major* parasites and compared the course of infection to that which occurred in the J_H strain of mice on the BALB/c background. J_H mice have a targeted deletion of the immunoglobulin heavy chain J locus and, therefore, make no antibody. Normal BALB/c mice are susceptible to Leishmania infections (12) and developed large lesions, as expected (Fig. 1, closed circles). The passive administration of polyclonal anti–*L. major* antiserum to wild-type BALB/c mice had essentially no effect on lesion progression (Fig. 1, open circles). These mice developed lesions with the same size and kinetics as mice that received no antisera. Furthermore, the two groups of BALB/c mice had similar numbers of parasites within their lesions at the conclusion of the observation period (Fig. 1, inset). However, infections of J_H mice with the same number of parasites resulted in a different outcome. J_H mice were relatively resistant to disease, developing modest lesions (Fig. 1, closed triangles) that contained several orders of magnitude fewer parasites (Fig. 1, inset). In three separate experiments, using a minimum of four mice per experimental group, the mean lesion size of infected J_H mice was 1.22 ± 0.31 mm at 35 d after infection, whereas infected BALB/c mice developed lesions that were 5.01 ± 0.43 mm. The passive administration of immune serum to J_H mice reversed this resistant phenotype and actually exacerbated disease, restoring lesion size to that observed in wild-type BALB/c mice (Fig. 1, open triangles). The number of parasites in these lesions was comparable to that observed in wild-type BALB/c mice (Fig. 1, inset).

A similar study was performed, except that anti–*L. major* antiserum was administered to J_H mice after the infection had progressed for 3 wk. This was done to determine the kinetics with which antibody to *L. major* could exacerbate infections (Fig. 2). In these studies, two groups of J_H mice were infected with *L. major* for 3 wk. One of the groups was given a single 200-μl i.p. injection of anti–*L. major* antiserum. Within 1 wk of antiserum administration, footpad lesions in this group were already significantly larger than the controls (Fig. 2). 3 wk after the injection of antiserum, the lesions had progressed to a point where their size (Fig. 2, open circles) and the number of parasites within them (Fig. 2, inset) was comparable to susceptible BALB/c mice. Similar studies were performed using IgG that was affinity purified from the immune serum used in Fig. 2. A single i.p. injection of 600 μg of purified α–*L. major* (αLm)-IgG into mice at 3 wk after infection caused a similar increase in lesion formation with similar kinetics as unfractionated immune serum

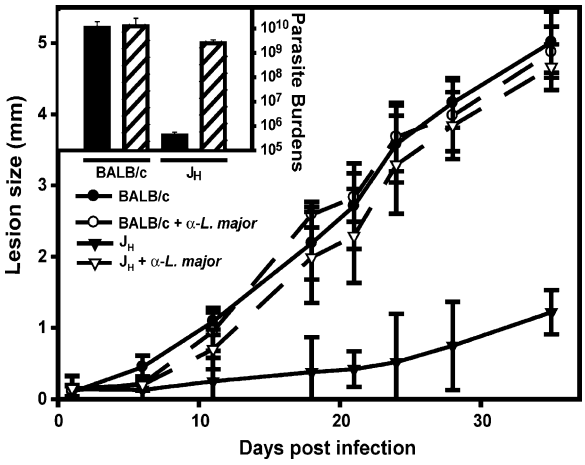


Figure 1. Serum reconstitution of J_H mice. Lesion size of J_H mice (closed triangles) and BALB/c mice (closed circles) was compared with J_H mice (open triangles) and BALB/c mice (open circles) given 200 μl of antisera to *L. major* on days 1, 7, and 14. Parasite burdens (inset) in BALB/c and J_H mice that were infected with (diagonally striped bars) or without (black bars) αLm (α–*L. major*) antisera were determined on day 35 by limiting dilution assays as described previously (reference 3). Error bars represent the standard error of the mean of three separate experiments done with a minimum of four mice per group.

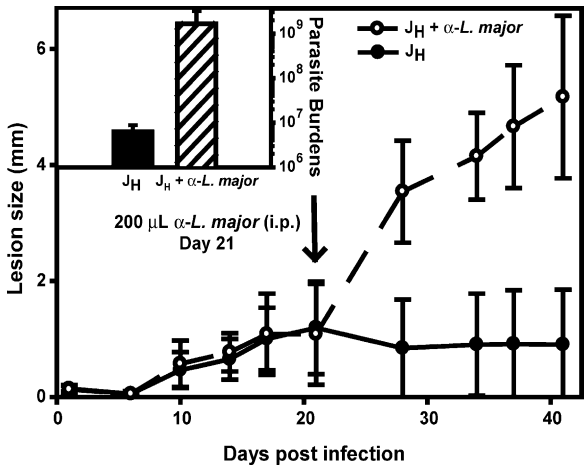


Figure 2. Serum reconstitution of J_H mice 3 wk after infection. Lesion size of J_H mice (closed circles) were compared with those of J_H mice given 200 μl αLm (α–*L. major*) antiserum on day 21 after infection (open circles). Parasite burdens (inset) were determined at 42 d after infection by limiting dilution assays as described previously (reference 3). Error bars represent the standard deviation of the mean of four determinations. This experiment is representative of three.

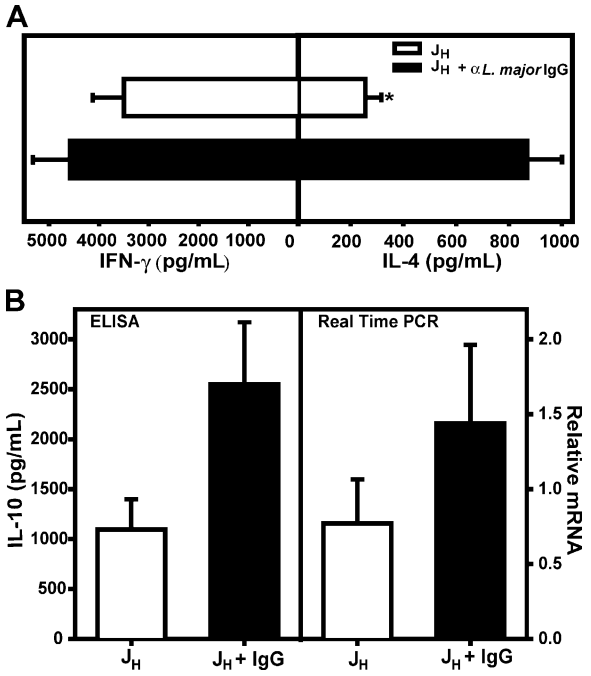


Figure 3. Cytokine production in mice administered anti–*L. major* antiserum. (A) Cytokine production by lymph node T cells from infected J_H mice (white bars) was compared with J_H mice administered 200 μl αLm (α–*L. major*) antiserum on days 1, 7, and 14 after infection (black bars). Lymph nodes were removed on day 21 and stimulated with soluble leishmania antigen. Supernatants were harvested 72 h later and assayed for IFN-γ and IL-4 by ELISA. (B) IL-10 production in lesions of J_H mice. IL-10 protein (left) and mRNA (right) were determined in two groups of J_H mice infected with 2×10^5 *L. major* amastigotes. On day 21 of infection, one group was administered 600 μg of purified αLm-IgG i.p. IL-10 protein levels in the lesions of three infected mice were measured by ELISA 4 d after the administration of αLm-IgG (left axis). RNA was isolated from footpad lesions on day 2 after IgG administration, and real-time PCR was performed to determine relative IL-10 mRNA (right axis). Levels represent the average from three infected mice, and mRNA was normalized to hypoxanthine phosphoribosyltransferase levels in a single infected foot.

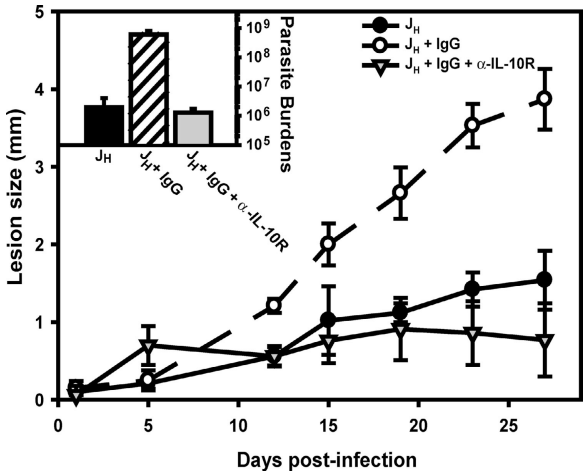


Figure 4. IgG reconstitution of J_H mice and the effect of α–IL-10R mAb. Three parallel groups of J_H mice were infected with 2×10^5 *L. major* amastigotes. One group (open circles) was administered 600 μg of purified αLm-IgG i.p. on days 1, 8, and 15. Another group (gray triangles) was given the same dose of αLm-IgG, along with α–IL-10R on days 0 (1 mg), 7 (200 μg), and 14 (200 μg). The third group (closed circles) received no treatment. Lesion size was measured at semi-weekly intervals. Parasite burdens (inset) were determined by limiting dilution. Error bars represent the standard deviation of the mean.

(Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20041470/DC1).

IL-10 production during Leishmania infection

Cytokine production from lymph node T cells was analyzed after infection of J_H mice with *L. major* in the presence or absence of antibody. The administration of αLm-IgG resulted in a significant increase in the production of IL-4 by stimulated lymph node T cells (Fig. 3 A), but little to no decrease in the production of IFN-γ levels by these cells. Thus, the presence of antibody to *L. major* correlates with an increase in IL-4 production by parasite-specific T cells. However, the alteration in the production of these T cell cytokines did not appear to be substantial enough to account for the large differences in lesion formation in these mice. Consequently, we examined a possible role for IL-10. We (3) and others (4, 5) have previously shown that mice lacking IL-10 were relatively resistant to leishmaniasis. We examined the production of IL-10 in the lesions of mice infected with Leishmania. IL-10 mRNA and protein were measured in infected feet 2 and 4 d, respectively, after the administration of αLm-IgG. J_H mice given a single dose of purified αLm-IgG i.p. produced approximately twice as much IL-10 protein and mRNA in their lesions, relative to parallel groups of mice infected at the same time, which did not receive IgG (Fig. 3 B).

To confirm a role for IL-10 in lesion progression, J_H mice were infected with *L. major* and administered purified αLm-IgG along with a monoclonal antibody to the IL-10 receptor (13). Infected J_H mice developed modest lesions (Fig. 4, closed circles), whereas those administered αLm-IgG developed large ulcerated lesions with high numbers of parasites (Fig. 4, open circles). These mice had to be killed due to excessive lesion progression. The administration of a monoclonal antibody to the IL-10 receptor completely prevented the exacerbation of disease caused by αLm-IgG (Fig. 4, open triangles). In fact, lesions in mice treated with αLm-IgG plus αIL-10R were essentially indistinguishable from uninfected feet by day 30 after infection (unpublished data).

To determine whether irrelevant immune complexes could have a similar effect on lesion progression, we immunized the genetically resistant C57BL/6 mice with OVA in IFA. 2 wk after a booster immunization, these mice had developed high titers of IgG antibody to OVA (unpublished data). They were then infected with *L. major* suspended in a solution of PBS containing 50 μg/ml OVA. Immunized

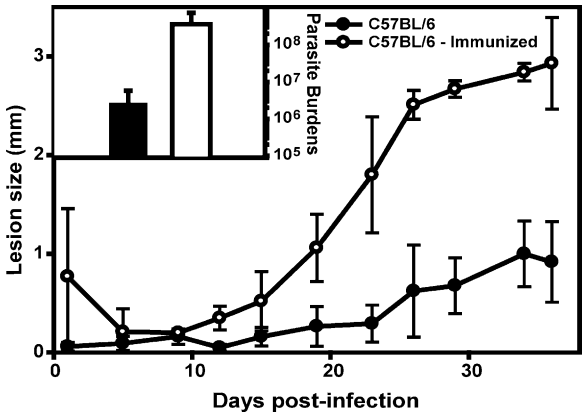


Figure 5. The immunization of C57BL/6 mice with OVA. Lesion development in OVA-immunized mice (open circles) was compared with control mice given IFA alone (closed circles). Immunized mice were given 25 μg OVA in 500 μL of IFA and boosted 2 wk later. Both groups of mice were infected with *L. major* resuspended in PBS containing 50 μg/ml OVA. Parasite burdens (inset) were determined on day 34. Error bars represent the standard deviation of the mean.

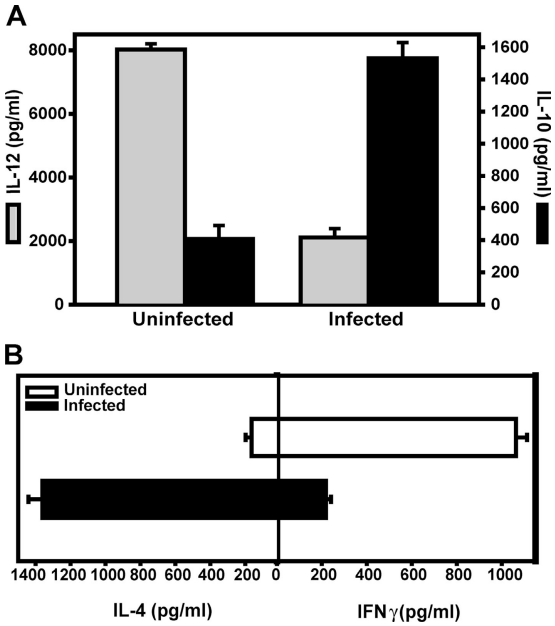


Figure 6. In vitro cytokine production. (A) Lesion-derived *L. major* amastigotes were added to bone marrow–derived macrophages in the presence of inflammatory LMW-HA. Supernatants were harvested 24 h later and cytokines IL-12 (gray bars) and IL-10 (black bars) were measured by ELISA. (B) The production of cytokines, IFN-γ and IL-4, from T cells was measured by ELISA 3 d after primary stimulation. OVA-specific TCR transgenic T cells were added to either uninfected (white bars) or *L. major*–infected (black bars) macrophages cultivated with OVA and LMW-HA.

mice making antibodies to OVA (Fig. 5, open circles), developed larger lesions with two logs more parasites (inset) compared with sham immunized mice receiving IFA alone and the same *L. major* infection (Fig. 5, closed circles).

In vitro studies on the mechanism of IgG-mediated immune enhancement

In vitro studies were performed to measure cytokine production by macrophages infected in vitro with lesion-derived *L. major* amastigotes (Fig. 6 A). To recreate the lesion environment, macrophages were cultivated with inflammatory low molecular weight hyaluronic acid (LMW-HA) before infection as described previously (3). Uninfected macrophages cultivated on LMW-HA produced relatively high levels of IL-12 (Fig. 6 A, gray bars) and only modest levels of IL-10 (Fig. 6 A, black bars). The addition of lesion-derived amastigotes to these cells caused a reciprocal change in cytokine production. Infected macrophages produced low amounts of IL-12 and relatively high levels of IL-10 (Fig. 6 A) as described previously, using soluble immune complexes (11).

To determine the extent to which Leishmania infection could influence APC function, macrophages were infected with lesion-derived amastigotes, pulsed with OVA, and used to present antigen to naive T cells from D011.10 mice, which carry an OVA-specific transgenic TCR (14). Uninfected macrophages cultivated with LMW-HA induced OVA-specific T cells to produce primarily IFN-γ when they were used as APCs (Fig. 6 B, white bar). However, macrophages infected with amastigotes preferentially induced the production of IL-4 from T cells, with a marked decrease in IFN-γ (Fig. 6 B, black bar). Thus, infection of macrophages with Leishmania amastigotes reverses their inflammatory cytokine profile, and this can influence T cell cytokine production.

IgG antibodies in human VL

In vitro infection studies were performed to measure IL-10 production from human monocytes infected with *Leishmania chagasi* amastigotes. For these studies, IgG-free axenically grown *L. chagasi* amastigotes were used to infect normal human monocytes. Axenic amastigotes induced little to no detectable IL-10 production from human monocytes (Fig. 7). However, incubating these organisms in serum from a patient with VL induced the production of high levels of IL-10 from monocytes (Fig. 7). Parallel groups of axenic amastigotes incubated in normal serum from uninfected volunteers failed to induce IL-10 production from monocytes (Fig. 7). Thus, serum from infected patients opsonizes amastigotes to induce the production of IL-10 from infected monocytes.

To relate these experimental observations to human VL, we examined a group of patients from Brazil who had bone marrow aspirate-confirmed VL. We sought to correlate the severity of VL with antibody levels and/or delayed-type hypersensitivity (DTH) responses. To do this, we examined a total of 317 patients with VL. These patients were selected at either the time of diagnosis or within ~1 yr of treatment and grouped together for this initial analysis. Of the total of 317 patients analyzed, 280 of them (88%) were antibody positive. This is in stark contrast with patients with cutaneous leishmaniasis, in which IgG antibody titers are typically low or absent (15). Of these 280 antibody-positive patients, 252 of

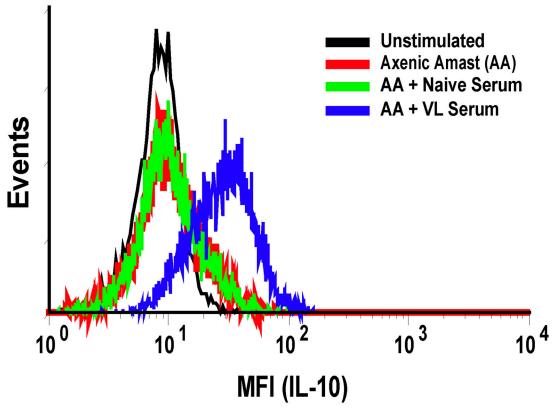
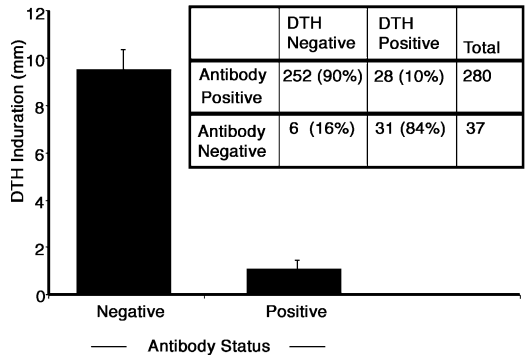


Figure 7. Flow cytometry to detect intracellular IL-10. Human monocytes were infected with a 10:1 ratio of axenic amastigotes of *L. chagasi*. Before infection, some amastigotes were incubated at 4°C for 15 min in 5% serum from either an uninfected volunteer (naive serum) or from a patient with visceral leishmaniasis (VL serum). Monocytes were incubated in Golgi stop for 2 h, fixed, and permeabilized and stained for intracellular IL-10 expression. Cells were gated on CD14 expression.

them (90%) were DTH negative. Conversely, of the 37 patients that were antibody negative, 31 of them (84%) were DTH positive, $P \leq 0.0001$ (Table in Fig. 8). The size of the DTH response was also calculated in these patients. Antibody-negative individuals had a mean induration score of 9.53 mm, whereas antibody-positive subjects had essentially no detectable DTH response, with an average induration of only 1.08 mm (Fig. 8). Thus, there was a strong negative correlation between antileishmanial IgG levels and DTH responses ($P = 0.007$).

A subset of these patients who were analyzed at the time of initial diagnosis were monitored at 6-mo intervals after



	DTH Negative	DTH Positive	Total
Antibody Positive	252 (90%)	28 (10%)	280
Antibody Negative	6 (16%)	31 (84%)	37

Figure 8. DTH and antibody responses in patients with visceral leishmaniasis (VL). Delayed-type hypersensitivity (DTH) reactions were measured in 317 bone marrow aspirate-confirmed VL patients. The mean induration size of the DTH response (in mm diameter) was measured at 48 h. (inset table) The same VL patients were analyzed for antibody and DTH responses. Antibody levels were judged to be positive if they were >3 SD above a mean control titer. DTH responses >5 mm in diameter were judged to be positive. This figure designates a significant negative association between DTH and antibody responses. Pearson χ^2, df = 1; $P \leq 0.0001$.

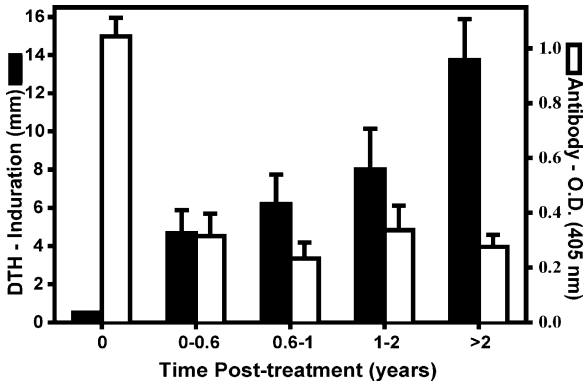


Figure 9. DTH and Leishmania-specific antibody responses in VL patients after treatment. The mean diameter induration score of the DTH response, measured in mm, is shown by the black bars (left), and the mean antibody titers, expressed as OD reading, are shown by the white bars (right) as a function of time after treatment (abscissa).

treatment. At the time of diagnosis, DTH responses were absent (Fig. 9, black bars) and antibody titers were high (Fig. 9, white bars). After treatment, DTH responses progressively increased over time, from <1 mm at the time of treatment to >6 mm after 1 yr of successful treatment (Fig. 9). Conversely, the mean antibody titers progressively declined over time from a mean of 1.219 (± 0.4753) to 0.246 (± 0.222) post-treatment (Fig. 9). Thus, in patients with VL, antibody levels correlate with, and are predictive of, active disease. Disease resolution after treatment parallels a decline in IgG and the development of DTH responses to leishmanial antigens.

DISCUSSION

We examined a role for IgG immune complexes in leishmaniasis, and conclude that IgG antibody not only fails to provide protection against this intracellular pathogen, but it can actually contribute to disease progression. A previous observation had shown that IgG could aggravate *Leishmania amazonensis* infections in mice (16), but did not identify a mechanism. We show the mechanism of this exacerbation is by inducing activated macrophages to produce IL-10 rather than IL-12. A role for IL-10 in leishmaniasis has been described previously (3–5), but the production of this cytokine has not been linked to the presence of IgG-containing immune complexes, nor have the cells producing this cytokine been definitively identified. Previous studies have identified a role for regulatory T cells in sustaining murine cutaneous leishmaniasis (17). These cells can produce IL-10, and regulatory T cells may be an important source of IL-10 during disease progression. In the present work, we show that infected macrophages can also produce IL-10 in response to the appropriate stimulation. In this case, the stimulation is host IgG, which combines with the parasite to form immune complexes. IL-10 produced by macrophages can exert both direct and indirect influences on disease progression. IL-10 is a potent inhibitor of macrophage activation, providing a direct way in which immune complexes can prevent parasite

eradication, even in the presence of an ongoing immune response. Immune complexes can also exert an indirect effect by influencing APC cytokine production to bias T cell responses (Fig. 6 B). In lesions, macrophages (18) and dendritic cells (19) are in close association with T cells, placing them in a prime position to influence T cell cytokine production. Immune complexes not only induce activated macrophages to produce IL-10 (Fig. 6 A), but they also induce both macrophages (9) and dendritic cells (20) to switch off their production of IL-12.

The observations made in the murine model of leishmaniasis were confirmed in patients with VL. We demonstrate that high antileishmanial antibodies in human VL correlate with peak parasitemia, and with negative DTH responses. Successful treatment resulted in decreased antibody titres and a restoration of DTH responses. Previous studies have identified immune complexes and rheumatoid factor to be high in patients with VL (21, 22). Polyclonal B cell activation and high antibody titers have been observed in canine as well as human VL (23–25). In many ways, the infection of BALB/c mice with *L. major* may be a better model for human VL than for cutaneous leishmaniasis. These mice develop high antibody titers, and the parasites frequently metastasize to distant locations including the bone marrow, liver, and spleen (26). Similar to human VL, BALB/c mice typically succumb to infection with *L. major*. Finally, the DTH response in these mice is smaller and more transient than in resistant mice (27). Interestingly, 20 yr ago it was demonstrated that the suppression of DTH responses in BALB/c mice required the presence of B cells (28). Thus, some of the observations made in the BALB/c model of infection with *L. major* can likely be extended to human VL.

In summary, we have identified a novel form of antibody-dependent immune enhancement (ADE), where immune complexes can influence cytokine production by APCs and prevent the eradication of intracellular pathogens. These observations may provide a general mechanism to explain ADE. The phenomenon of ADE has been observed in Dengue hemorrhagic fever (29), where the presence of antibody causes a marked increase in viremia and viral pathogenesis. Intracellular bacteria may also exploit IL-10 as a mechanism to establish disease. Recent studies have associated the production of IL-10 from macrophages with the progression of lepromatous leprosy (30). Finally, these observations may also strike a cautionary note pertaining to the development of antibody-based vaccines against intracellular pathogens (31, 32). The present studies would suggest that antibody-mediated immune enhancement, resulting in IL-10 production from macrophages, should be considered as a potential risk for some vaccination protocols.

MATERIALS AND METHODS

Patient population. These studies were reviewed and approved by the Universidad Federal do Rio Grande do Norte Ethical Committee (CEP-UFRN 19/01), by the Brazilian National Ethical Committee of Research (CONEP 4572), and by the IRB of the University of Maryland. Serum was obtained from patients with parasitological confirmed cases of VL. Antileishmanial antibodies were determined as described previously (33). The cutoff for a positive titer was 3 SD above the mean value of control sera, obtained from 30 uninfected unexposed individuals (34). Each sample was analyzed in triplicate, and positive and negative controls were included in all assays. The DTH response in these individuals was performed by intradermal injection of 25 μg of *L. chagasi* protein provided by the Infectious Disease Research Institute as described previously (35). Montenegro skin tests (DTH) were read after 48 h and scored as positive if the induration exceeded 5 mm in diameter. The means of antibody and DTH responses were analyzed by Student's *t* test for dependent groups. To examine the influence of time and antibody on DTH response of patients with ongoing VL or in the process of recovering, a factorial two-way analysis of variance was used.

Animal studies. These studies were reviewed and approved by the University of Maryland Institutional Animal Care and Use Committee. BALB/c mice were purchased from the National Cancer Institute Charles River Laboratories and from Taconic. J_H mice were purchased from Taconic. DO11.10 mice (14), which have a transgenic T cell receptor (TCRαβ) for $OVA_{323-339}$, were purchased from The Jackson Laboratory and used as a source of antigen-specific $CD4^+$ T cells.

Antibody production and administration. Purified IgG against *L. major* was obtained by passing serum from infected BALB/c mice through the protein G column on a GradiFrac System (Amersham Biosciences). Purified α–IL-10R monoclonal antibody (36) was provided by K. Moore (DNAX Research Institute, Palo Alto, CA). IgG and α–IL-10R treatments were administered by i.p. injections at designated times during the experiment.

Parasites, infection, and parasite quantitation. Lesion-derived *L. major* amastigotes (WHO MHOM/IL/80/Friedlin) were isolated from infected BALB/c mice as described previously (37). *L. chagasi* were obtained from a Brazilian patient with VL. Axenic *L. chagasi* amastigotes were grown as described previously (38). Mice were injected in the right hind footpad with either 2×10^5 or 2×10^6 *L. major* amastigotes. Lesion size was determined by using a caliper to measure the thickness of the infected footpad and subtracting the thickness of the contralateral uninfected footpad as described previously (3). Parasite burdens were determined by a serial dilution of single cell suspensions made from excised lesions as described previously (3).

Cytokine production by infected macrophages. Macrophages were cultured overnight in 24-well plates at a density of 2×10^5 cells/well. Cells were stimulated with 150 μg/ml LMW-HA (Worthington) before the addition of a 10:1 ratio of lesion-derived (IgG positive) *L. major* amastigotes. 24 h after infection, supernatants were collected and IL-10 and IL-12 levels were measured by ELISA as described previously (11). For opsonization of amastigotes with IgG, axenically grown *L. chagasi* amastigotes were incubated for 20 min at 4°C in nonimmune or immune serum from a patient with parasite-confirmed VL.

IL-10 production by human monocytes infected with *L. chagasi* amastigotes was measured by flow cytometry. Human monocytes were infected with a 10:1 ratio of serum opsonized *L. chagasi* amastigotes in the presence of 10 ng/ml LPS. After 15 min, monolayers were treated with Golgi stop (BD Biosciences) for an additional 2 h. IL-10 was detected using a PE rat α–mouse IL-10 monoclonal antibody (BD Biosciences).

IL-10 quantitation. IL-10 protein and mRNA levels were measured in infected feet after the administration of αLm-IgG. J_H mice were infected with 2×10^5 *L. major* amastigotes in the right hind footpad. 21 d after infection, mice were given a single i.p. injection of 600 μl αLm-IgG. Control J_H mice remained untreated. mRNA levels were measured by real-time PCR. Total RNA was extracted from the footpad lesions 48 h after treatment with α-Leishmania IgG, using the RNAqueous-4PCR kit obtained from Ambion. IL-10 levels were detected by real-time PCR. Real-time PCR was performed using the ABI PRISM 7700 Sequence Detection System and SYBR

green core PCR reagents (Applied Biosystems). The murine IL-10 primer sequences used for this analysis were as follows: sense, 5′-CCACAAAGCAGCCTTGCA-3′ and antisense, 5′-AGTAAGAGCAGGCAGCATAGCA-3′. The hypoxanthine phosphoribosyltransferase primers used were as follows: sense, 5′-GTTGGATACAGGCCAGACTTTGTTG-3′ and antisense, 5′-AGGGTAGGCTGGCCTATAGGCT-3′. IL-10 protein levels were measured by ELISA. Lesions were homogenized in lysis buffer (15 mM Tris-HCl, 0.5% Triton X-100, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$) as described previously (39). Samples were incubated on ice for 30 min and centrifuged at 850 *g* for 30 min. Supernatants were analyzed by ELISA.

T cell cytokine production. $CD4^+$ T cells were prepared from the spleens of DO11.10 mice by immunomagnetic negative depletion as described previously (11). For T cell stimulation assays, 2×10^5 macrophages were primed overnight with 100 U/ml rIFN-γ (R&D Systems). Cells were washed and stimulated with 150 μg/ml LMW-HA in the presence of 150 μg/ml OVA (Worthington). Fresh amastigotes of *L. major* were isolated from active lesions in BALB/c mice, washed, and added to some of the macrophages at a 10:1 ratio. 5×10^5 $CD4^+$ T cells were added to each well 2 h later, in a total volume of 0.6 mL RPMI 1640 (Cellgro) supplemented with 10% FCS, Hepes, penicillin/streptomycin, and β-mercaptoethanol. Primary T cell cytokine measurements were made by ELISA at 72 h as described previously (11).

Cytokine production by lymph node T cells was determined by ELISA. In brief, mice were infected with *L. major* in the presence or absence of αLm-IgG as described before. At day 21 after infection, lymph nodes were removed, and unfractionated lymph node cells were stimulated with 50 μg/ml soluble leishmania antigen. Supernatants were collected 72 h later, and IFN-γ and IL-4 were measured by ELISA.

Immunization with OVA. C57BL/6 mice were immunized against OVA by giving 25 μg OVA in 500 μL of IFA (Difco Laboratories) by i.p. injection. A control group was given IFA alone. 2 wk after the initial injection the mice received a "booster" dose of 25 μg OVA in 500 μL of IFA. 2 wk after receiving the second dose of OVA, some of the mice were bled to confirm the presence of anti-OVA IgG. The rest of the littermates were infected in the right hind footpad with 2×10^6 *L. major* amastigotes resuspended in PBS containing 50 μg/ml OVA.

Online supplemental material. Disease progression in J_H mice treated with αLm-IgG. Three groups of J_H mice (five per group) were infected with equal amounts of *L. major* amastigotes. At 20 d after infection, one group of mice received 200 μl of antisera from infected BALB/c mice and a second group received 600 μg purified IgG that was isolated from this antiserum. The third (control) group received saline. Footpad swelling was measured over the next 3 wk as described before. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20041470/DC1.

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