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Interleukin-4 but Not Gamma Interferon Production Correlates with the Severity of Murine Cutaneous Leishmaniasis

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For murine cutaneous leishmaniasis, data to date suggest a correlation between the presence of gamma interferon (IFN-γ) and resistance in C57BL/6 mice and the presence of interleukin-4 (IL-4) and disease in BALB/c mice. In this study, 13 inbred strains of mice covering the range of susceptibility to disease were infected with *Leishmania major* to determine whether the subsequent expression of IFN-γ or IL-4 is a reliable indicator of cure or progressive disease. The presence of IL-4 and IFN-γ mRNAs in the draining lymph nodes was examined 9 weeks after infection, when differences in disease severity became obvious. There were large differences in the levels of IL-4 mRNA among the different strains, whereas IFN-γ mRNA was detected at similar levels in all strains. The levels of IL-4 mRNA correlated with lesion score, with susceptible and intermediate strains containing up to 100-fold more than any of the resistant strains. Differences in the levels of IFN-γ mRNA were within only a fourfold range, with significant overlap among susceptible, intermediate, and resistant strains. Similarly, the levels of IFN-γ secreted in vitro by lymph node cells from infected mice in response to *L. major* antigens were within a 10-fold range for most strains, and there was no correlation with lesion score. Analysis of *Leishmania*-specific antibody levels revealed a correlation between immunoglobulin G1 (IgG1) titers and lesion score, consistent with the role of IL-4 as a switch factor for IgG1. In contrast, there was no correlation between IgG2a titers and lesion score, supporting the notion that IFN-γ synthesis (which promotes IgG2a production) is not correlated with disease state. These data suggest that along the spectrum of murine cutaneous leishmaniasis, IL-4 is a reliable indicator of disease, but IFN-γ is not prognostic for resistance.

Infection of mice with *Leishmania major* has been used widely as an experimental model system for the study of human leishmaniasis (46). The majority of inbred strains of mice develop small lesions that heal (resistant strains), while a few strains develop large lesions and fatal visceralizing infections (susceptible strains). Mice of certain strains, such as A/J, show variability in their susceptibility to infection with *L. major*, with some mice in the group being cured and others not (intermediate strains), a phenomenon that may reflect a combination of genetic and environmental factors (8). Most studies on the immune mechanisms responsible for resistance or susceptibility have made use of two inbred strains of mice that are genetically susceptible (BALB/c) and resistant (C57BL/6) to chronic disease. The divergent outcomes following infection with *L. major* in these mice correlate with the relative abundances of particular lymphokine mRNAs in draining lymph nodes and spleens (10, 11). Thus, BALB/c nonhealing mice produce interleukin 4 (IL-4) and IL-10 and lower levels of gamma interferon (IFN-γ), whereas the reciprocal pattern is found in C57BL/6 healing mice late in infection. On the basis of these and earlier data, it has been suggested that the prognosis of a *Leishmania* infection can be determined by analyzing the lymphokines produced by *Leishmania*-specific cells from infected mice and humans (5, 19, 27, 31, 36). While lymphokine profiles in BALB/c and C57BL/6 mice have been subjected to intensive investigation, very little is known about lymphokine responses to *L. major* infections in other strains of mice. This information would show further whether IFN-α and IL-4 are indeed reliable indicators of cure and progressive disease, respectively. In this study, we showed that for 13 strains of mice that differ in their susceptibility to *L. major*, only IL-4 levels correlated with disease severity, suggesting that in murine cutaneous leishmaniasis, IL-4 is a reliable indicator of disease, but IFN-γ is not prognostic for resistance.

MATERIALS AND METHODS

Mice. A/J, BALB/c, BALB.G, BALB.K, BALB/c × C57BL/6J (B/c × C57), B10.A(4R), CBA/N, CBA.bg, CB.17, C57BL/6J, C57BL/6bg, C57B10pdW^f × DBA/2W^f (W^f/W^f F1), and NZW mice were bred at The Walter and Eliza Hall Institute of Medical Research under specific-pathogen-free conditions and maintained conventionally. All mice used were female, aged 7 to 12 weeks. BALB/c *nu/nu* (hypothymic) mice were used for in vivo passaging of *L. major* parasites.

Parasites. Virulent *L. major* line V121 (9) was cloned from the original LRC-L137 stock obtained from the World Health Organization Reference Centre for Leishmaniasis, Jerusalem, Israel. Amastigotes were harvested from skin lesions of BALB/c *nu/nu* mice by use of a rapid protocol developed by Glaser et al. (6). Groups of eight mice were injected with 2×10^6 amastigotes intradermally at the base of the tail. Lesion development was monitored weekly and

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assigned a score from 1 to 4 (where 1 represents a small localized swelling and 4 represents a large disseminating lesion) as detailed previously (20).

Media and reagents. Cells were cultured in modified DME (14) supplemented with 216 mg of L-glutamine per liter, 5×10^{-5} M 2-mercaptoethanol, and 5% fetal calf serum (Flow Laboratories, North Ryde, Australia) (DME–5% FCS) in a humidified atmosphere of 5% CO_2 in air. The culture supernatant from cells transfected with mouse IL-4 cDNA (13) (a gift from F. Melchers, Basel Institute for Immunology, Basel, Switzerland) was used as a source of recombinant IL-4. Recombinant IFN-γ was purchased from Genentech Inc., San Francisco, Calif. Soluble leishmanial antigen (SLA) was prepared with V121 promastigotes grown in Schneider's drosophila medium (GIBCO BRL Life Technologies, Inc., Penrose, Auckland, New Zealand) containing 10% fetal calf serum as described previously (25).

RNA preparation. The draining lymph nodes (inguinal and lower periaortic) from at least three mice per group were pooled and homogenized in 6 M guanidine isothiocyanate–25 mM citrate–0.5% sodium lauroylsarcosinate–50 mM 2-mercaptoethanol by use of a Dounce homogenizer. RNA was purified from the homogenate as described previously (18).

S1 nuclease protection analysis. S1 nuclease protection experiments were performed essentially as described by Berk and Sharp (4) with 65 μg of total RNA or yeast tRNA (Boehringer GmbH, Mannheim, Germany) and ^{32}P-end-labeled probes for IFN-γ (44) or glyceraldehyde-phosphate dehydrogenase (GAP-DH) (30). Before electrophoresis, the samples were denatured in 200 μl of 0.3 M NaOH–1 mM EDTA at 65°C for 10 min and neutralized by the addition of 20 μl of 3 M HCl–10 mM Tris (pH 7.6)–1 mM EDTA. Five micrograms of carrier tRNA was added to the samples, and nucleic acids were precipitated with ethanol. Protected fragments were separated on a 5% polyacrylamide sequencing gel and quantitated on a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.) by use of ImageQuant software (Molecular Dynamics). Relative signal intensities were calculated after background subtraction and normalization of the total mRNA content of each sample on the basis of the GAP-DH mRNA content. The amount of IFN-γ mRNA detected in RNA from infected BALB/c mice was arbitrarily set at 10 U.

PCR analysis. Synthesis of cDNA, the polymerase chain reaction (PCR), and PCR oligonucleotide primers for IL-4 and IFN-γ have been described (18, 43). PCR oligonucleotide primers for β-actin were as follows: 5′ primer, GACAT GGAGAAGATCTGGCA; 3′ primer, GGTCTTTACGGATG TCAACG. All primer pairs were included in each reaction. Quantitation of relative levels of mRNA was performed by an adaptation of the procedures originally described by Rappolee et al. (32–34) with modifications (21). In brief, six fourfold serial dilutions of each cDNA sample were subjected to PCR amplification; serial dilutions of cloned cDNAs starting at an input dose of 10^{-15} g were used as a standard for the quantitation of sample cDNAs. The PCR products were analyzed by Southern blotting as described previously (18, 43), and the amount of a cDNA-specific product generated was determined by use of ImageQuant software on a PhosphorImager. Plots of the logarithm of PCR product yield versus the logarithm of cDNA input for each sample were compared with a standard curve generated by use of cloned cDNA to quantitate the levels of specific cDNAs in the starting samples. Lymphokine transcript levels were determined after normalization to the level of the constitutively expressed β-actin transcript to compensate for intersample differences in RNA integrity, cDNA synthesis, or PCR efficiency. Reverse transcription reaction and PCR mixtures containing *Escherichia coli* tRNA were included as negative controls.

Bulk lymph node cell cultures. The draining lymph nodes from two animals per group (or individual mice as indicated) were harvested and pooled. Single-cell suspensions were prepared by forcing tissues through a fine wire mesh and washing them twice by centrifugation. Cells (2×10^6) were cultured in 1 ml of DME–5% FCS in 48-well plates (Costar, Cambridge, Mass.) in the presence or absence of antigen (25 μg of SLA per ml). Supernatants were harvested 24 h later for lymphokine analysis.

Lymphokine assays. IL-4 and IFN-γ were assayed with the specific lymphokine-responsive cell lines CT.4S and WEHI-279 as described previously (25). Tissue culture supernatants containing anti-IL-4 monoclonal antibody 11B11 (28) or neutralizing anti-IFN-γ monoclonal antibody R4-6A2 (ATCC HB170) (40) were used to confirm the specificity of these assays.

Antibody titers. Pooled sera from two mice per group (or individual mice as indicated) were assayed for anti-*L. major* immunoglobulin G1 (IgG1) and IgG2a antibodies by an isotype-specific enzyme-linked immunosorbent assay (ELISA) (22). Round-bottom 96-well polyvinyl chloride plates (Dynatech, Chantilly, Va.) were coated with 50 μg of SLA per ml, and the remaining reactive sites were blocked with phosphate-buffered saline containing 5% skim milk plus 0.05% Tween 20. Serially diluted sera were added at room temperature, and horseradish peroxidase-conjugated goat anti-mouse IgG1 or IgG2a (Southern Biotechnology Associates, Birmingham, Ala.) was added 1 h later. Bound antibodies were detected with ABTS [2,2′-azino-di-(3-ethylbenzothiazoline-6 sulfonic acid)] (Pierce, Rockford, Ill.). Titers were determined from the linear portion of parallel dose-response curves and were the reciprocal of the dilution at which samples showed an optical density reading of 0.7.

RESULTS

Infection of mice with *L. major*. Mice of 43 different inbred, congenic, and hybrid strains were injected intradermally at the base of the tail with *L. major* V121 amastigotes, and lesion development was monitored for 9 weeks. The majority of mice developed lesions that resolved at different times over the 9 weeks, while mice from a few strains failed to heal their lesions. Thirteen different strains that covered this spectrum of susceptibility were selected for further analysis. BALB/c, BALB.G, CB.17, and NZW were all highly susceptible to infection, and all mice in each group developed large lesions by 9 weeks postinfection. Among the resistant strains, CBA/N and CBA.bg were the most resistant, showing little or no effect at the injection site, whereas C57BL/6 and C57BL/6bg developed small lesions (mean lesion score, 1) that healed over a period of 6 to 8 weeks. Five strains [A/J, BALB.K, B/c × C57, B10.A(4R), and W^f/W^f F1] showed intermediate resistance; the majority of mice showed lesion cure over 6 to 8 weeks, but a few (one to four of eight mice) failed to heal their lesions over a 3-month period. This variation in lesion score among strains showing intermediate resistance was noted in three separate experiments. The mean lesion scores in mice of these strains were higher at the peak of the acute phase (3 to 6 weeks postinfection) than were those in mice of resistant strains (data not shown). These characteristics were taken to indicate that these strains lay between the resistant and the susceptible and

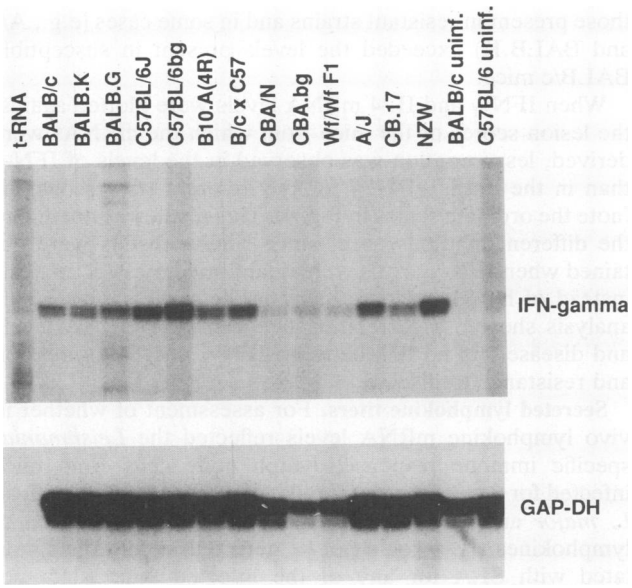

FIG. 1. IFN-γ mRNA levels among 13 mouse strains infected with *L. major*. S1 nuclease protection analysis was performed on total RNA from draining lymph nodes 9 weeks postinfection by use of probes for IFN-γ and GAP-DH. Total RNA from lymph nodes of uninfected (uninf.) BALB/c and C57BL/6 mice and tRNA served as controls.

were therefore termed intermediate. Despite the curing of lesions in some mice showing intermediate resistance, these mice continued to show signs of ongoing disease, as evidenced by enlarged lymph nodes up to 7 months later.

All susceptible, resistant, and intermediate strains of mice were analyzed 9 weeks after infection, since this time point corresponded to three different stages of infection; i.e., primary lesions had healed completely, were about to heal, or had failed to heal and remained as open wounds until the animals were sacrificed.

Lymphokine mRNA analysis. S1 nuclease protection experiments were performed to quantitate the levels of IFN-γ mRNA present in the draining lymph nodes of mice at 9 weeks postinfection. Infected mice of all strains expressed IFN-γ mRNA at elevated levels compared with uninfected BALB/c and C57BL/6 mice (Fig. 1). The expression of IFN-γ mRNA varied approximately fourfold between BALB/c mice, which expressed the lowest level detected, and C57BL/6 mice, which expressed some of the highest levels detected. BALB/c-related strains (BALB/c, BALB.K, BALB.G, CB.17, and B/c × C57) tended to express less IFN-γ mRNA; however, there was a considerable overlap in the range of IFN-γ levels expressed by susceptible, intermediate, and resistant strains. Highly resistant strains, e.g., CBA/N, expressed levels of IFN-γ mRNA similar to those expressed by the highly susceptible strains of the BALB/c background, and susceptible NZW mice or mice showing intermediate resistance, e.g., B10.A(4R), expressed IFN-γ mRNA at levels approaching or exceeding those detected in resistant C57BL/6 mice.

The relative levels of IL-4 mRNA were determined by an adaptation of the PCR. This method, which is more sensitive than S1 nuclease analysis, was required to detect the relatively low abundance of IL-4 transcripts in vivo in resistant strains. Preliminary studies demonstrated that serial dilutions of cDNA and of total RNA prior to cDNA synthesis yielded similar results (data not shown). Therefore, we, like others (21, 45), used serial dilutions of cDNA in the remainder of our experiments. Figure 2 shows that there was a broad range of IL-4 mRNA levels among the strains analyzed. The PCR used was shown to be linear over a >1,000-fold range of dilutions and was able to detect $<10^{-17}$ g of cloned IL-4 cDNA (Fig. 3). The fact that plots of the logarithm of PCR product yield versus the logarithm of cDNA input were linear (r^2 = 0.97; standard deviation = 0.06) and approximately parallel (slope = 0.80; standard

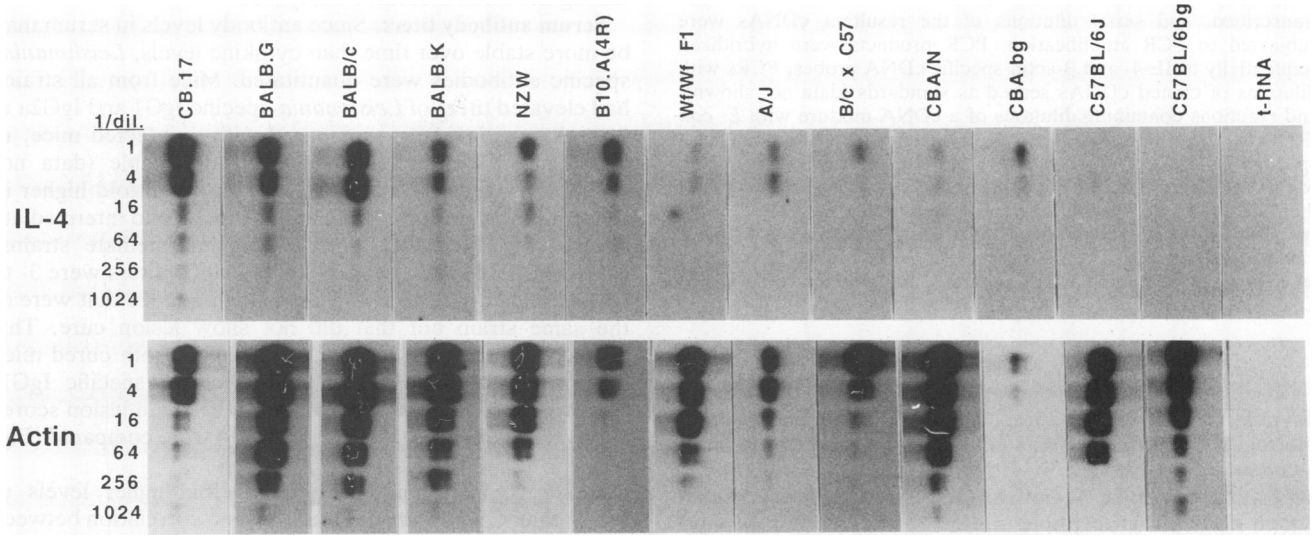

FIG. 2. Relative levels of IL-4 mRNA in mice infected with *L. major*, as determined by a modification of the PCR. Total RNA (1 μg) was reverse transcribed, and the PCR was performed on serially diluted cDNA with primers specific for IL-4 or β-actin. PCR products were hybridized sequentially to IL-4- and β-actin-specific cDNA probes. Dilutions of an IL-4-specific cDNA clone served as positive controls (data not shown). tRNA and cDNA prepared from uninfected C57BL/6 mice (data not shown) were negative.

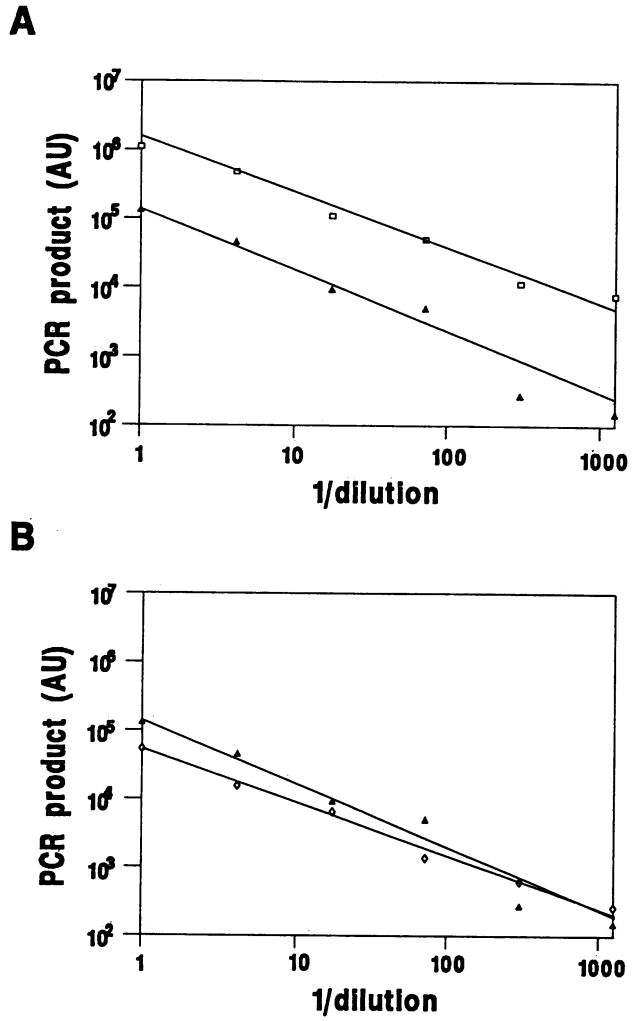

FIG. 3. PCR analysis of IL-4 mRNA in mice infected with *L. major*. One-microgram aliquots of total RNA extracted from the draining lymph nodes of *Leishmania*-infected mice were reverse transcribed, and serial dilutions of the resultant cDNAs were subjected to PCR amplification. PCR products were hybridized sequentially to IL-4- and β-actin-specific cDNA probes. PCRs with dilutions of cloned cDNAs served as standards (data not shown), and reactions containing dilutions of a cDNA mixture with *E. coli* tRNA as a template served as negative controls. The amount of a specific IL-4 PCR product in each of the bands shown in Fig. 2 (top panel) was determined by PhosphorImager analysis. The inverse of the dilutions of the cDNA samples was plotted against the yield of specific IL-4 PCR products. A comparison of these plots with the cloned cDNA standard curve was used to quantitate the IL-4 cDNA level in each sample. (A) □, BALB/c; ▲, IL-4 cDNA. (B) ◇, C57BL/6J; ▲, IL-4 cDNA. AU, arbitrary units.

deviation = 0.18) for all samples allowed the quantitation of the relative amounts of IL-4 cDNA in the samples. β-Actin levels were quantitated by similar means and used for the normalization of IL-4 expression. Our data showed that lymph nodes of susceptible strains of mice contained very high levels of IL-4 mRNA, while those of resistant strains (C57BL/6 and C57BL/6bg) contained low or barely detectable levels of IL-4 mRNA. All intermediate strains of mice expressed levels of IL-4 that were consistently higher than those present in resistant strains and in some cases (e.g., A/J and BALB.K) exceeded the levels present in susceptible BALB/c mice.

When IFN-γ and IL-4 mRNA levels were plotted against the lesion scores of the mice from which the mRNAs were derived, less variation was observed in the levels of IFN-γ than in the levels of IL-4 for the different strains of mice (note the ordinate scales in Fig. 4). This result was not due to the different methods used, since similar results were obtained when IFN-γ levels were quantitated by PCR analysis instead of by S1 nuclease analysis (data not shown). This analysis showed a correlation between IL-4 mRNA levels and disease and no link between IFN-γ mRNA expression and resistance to disease.

Secreted lymphokine titers. For assessment of whether in vivo lymphokine mRNA levels reflected the *Leishmania*-specific immune response, lymph node cells from mice infected for 9 weeks were stimulated in vitro for 24 h with an *L. major* antigen (SLA) and assayed for the secretion of lymphokines. IL-4 could not be detected in cultures stimulated with SLA for any of the infected mice (data not shown). IFN-γ titers were four- to fivefold higher in cultures from C57BL/6 mice than in those from BALB/c mice (Fig. 4c). Except for BALB.G mice, which secreted the lowest titers of IFN-γ, titers for all mice lay within a 10-fold range between BALB/c and C57BL/6bg. While susceptible mice secreted some of the lowest titers of IFN-γ, cells from some resistant and intermediate strains of mice that showed lesion cure (e.g., CBA.bg, W^f/W^f F1, and B/c × C57) secreted similarly low titers. An analysis of mice of intermediate strains, some of which did not show lesion cure by 9 weeks postinfection, showed that the levels of IFN-γ secretion depended on the genotype of the mouse and not on the presence or absence of lesions. Thus, cells from mice with lesion scores higher than 3 secreted the same amount of IFN-γ as cells from mice that were of the same strain and that were from the same box but that did not show lesion cure (Fig. 4c). Therefore, there was no correlation between lesion scores and *Leishmania*-specific IFN-γ secretion levels among the strains of mice analyzed.

Serum antibody titers. Since antibody levels in serum may be more stable over time than cytokine levels, *Leishmania*-specific antibodies were quantitated. Mice from all strains had elevated titers of *Leishmania*-specific IgG1 and IgG2a at 9 weeks postinfection, compared with uninfected mice, in which specific antibodies were not detectable (data not shown). However, IgG1 titers were up to 50-fold higher in susceptible strains of mice than in resistant and intermediate strains (Fig. 4d). For three of the intermediate strains, BALB.K, B10.A(4R), and W^f/W^f F1, IgG1 titers were 3- to 10-fold higher in mice with lesions than in mice that were of the same strain but that did not show lesion cure. This dichotomy was less marked for A/J mice: both cured mice and mice with lesions had high levels of specific IgG1. Nevertheless, there was a correlation between lesion scores and IgG1 titers when all strains of mice were compared (Fig. 4d).

While BALB/c mice showed fivefold-higher levels of IgG2a than C57BL/6 mice, there was no correlation between lesion scores and IgG2a titers for the 13 strains of mice analyzed. In particular, mice of the same genotype had similar IgG2a titers, irrespective of the presence or absence of lesions (data not shown).

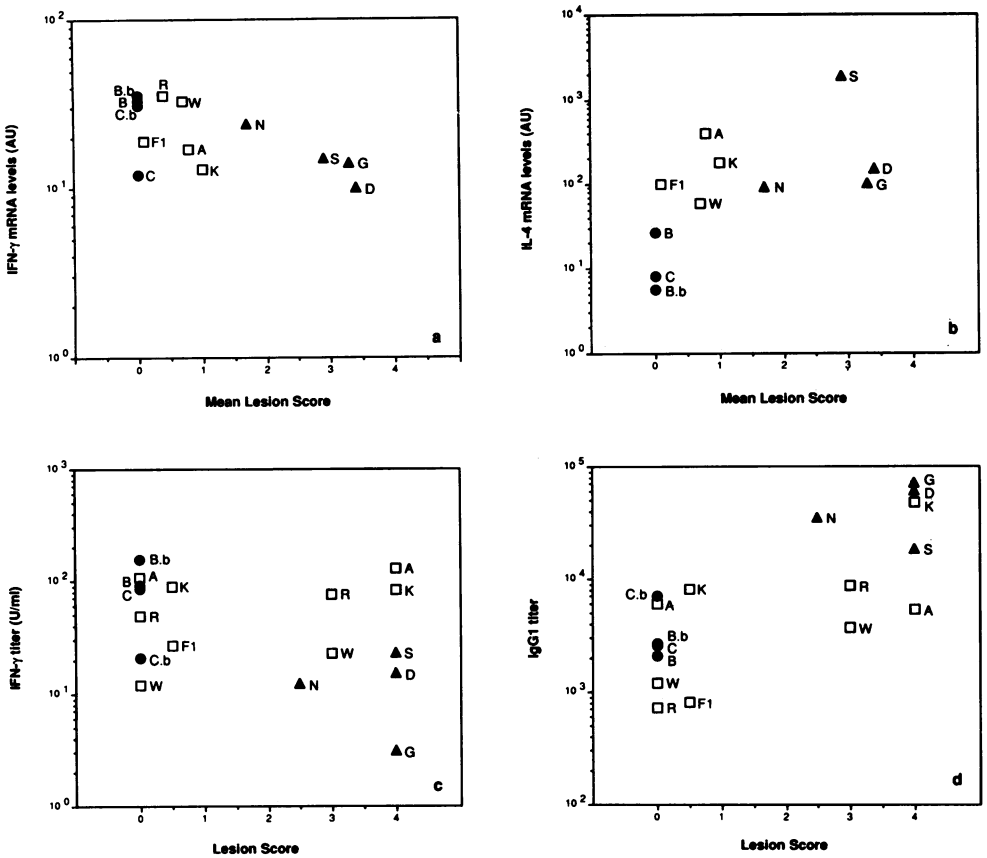

FIG. 4. Comparison of IFN-γ mRNA levels (a), IL-4 mRNA levels (b), secreted IFN-γ titers (c), and IgG1 serum antibody titers (d) with lesion scores for susceptible (▲), intermediate (□), and resistant (●) strains of mice. Levels of IFN-γ and IL-4 mRNAs in arbitrary units (AU) were calculated from the S1 nuclease protection (Fig. 1) and quantitative PCR (Fig. 2) analyses, respectively. IL-4 mRNA data for mice of strains CBA.bg and B10.A(4R) were not included because of the low levels of actin control mRNA obtained (Fig. 2). Titers of IFN-γ secreted by lymph node cells in response to *L. major* antigens were measured with an IFN-γ-responsive cell line. *L. major*-specific IgG1 titers were determined by an ELISA. Abbreviations: A, A/J; B, C57BL/6J; B.b, C57BL/6bg; C, CBA/N; C.b, CBA.bg; D, BALB/c; F1, B/c × C57; G, BALB.G; K, BALB.K; N, NZW; R, B10.A(4R); S, CB.17; W, W^f/W^f F1. Mean lesion scores for panels a and b were calculated from the average scores for eight mice. Lesion scores for panels c and d were based on the scores for the two mice used, except for A, K, and R, for which only single mice with lesions were available in this particular experiment.

DISCUSSION

The purpose of this study was to determine whether the levels of IFN-γ and IL-4 expression in mice infected with *L. major* are reliable and general indicators of cure and disease, respectively. Our results show that there was no correlation between IFN-γ production and resistance to disease for a large panel of inbred mice showing various degrees of susceptibility to disease. In contrast, IL-4 mRNA was consistently expressed at much higher levels in mice with chronic or ongoing disease, and the presence or absence of this lymphokine was therefore considered diagnostic of disease.

BALB/c and C57BL/6 mice have been used extensively in studies of leishmaniasis because of their innate susceptibility and resistance, respectively, to disease caused by *L. major*. These mice show differences in both cellular and humoral immune responses that have been attributed to the expansion of subsets of helper T cells secreting different lymphokines (10, 37). Our studies show that the differences observed between these two strains represent opposite ends of the range of responses seen for a group of 13 mouse strains. These 13 strains were selected because they covered the full spectrum of disease caused by *L. major* and included highly resistant, intermediately resistant, and highly susceptible mice. H-2 congenic mice and *bg* (beige) mutant mice, which are deficient in natural killer cells (the only other non-T-cell source of IFN-γ in vivo [7]), were also included. Differences in the *H-2* and *bg* mutations, however, were found not to influence significantly the *Leishmania*-induced lymphokine profiles. All mice were analyzed at 9 weeks postinfection. By this time, differences in the severity of disease (lesion score) were obvious between and within strains. Sampling of the different strains at 9 weeks therefore represented a cross-section of murine cutaneous leishmaniasis, similar to the study of one strain at different times during an infection (25). In highly resistant mice, e.g., CBA/N, the low levels of lymphokine mRNA may be due to the small numbers of parasites as a result of an effective cell-mediated response. However, since parasites persist in all strains of mice following healing of lesions (1, 15, 26), residual parasites would provide for continuous stimulation of a specific immune response. The in vitro restimulation of antigen-specific cells was an attempt to compensate for the presumably lower antigen-driven T-cell stimulation in vivo in these mice. In

agreement with the mRNA data, the levels of IFN-γ secreted in vitro did not correlate with lesion score either between strains or within a given strain. In contrast, at 9 weeks postinfection, a strong correlation between IL-4 expression and disease was maintained for all strains studied. Titers of *Leishmania*-specific IgG1 correlated with lesion score, consistent with the role of IL-4 as a switch factor for IgG1 (39), whereas there was no correlation between IgG2a titers (IgG2a production is promoted by IFN-γ [39]) and lesion score.

Studies in vivo and in vitro have demonstrated a role for IFN-γ in resistance to an *L. major* infection (3, 10, 36, 41), although other lymphokines, such as tumor necrosis factor alpha (TNF-α), have also been implicated (42). Reduced levels of TNF-α due to a polymorphism in the TNF-α locus of NZW mice (12) may account for the susceptibility of these mice to an *L. major* infection. Nevertheless, our finding that susceptible mice, including BALB/c mice, and mice of intermediate susceptibility with ongoing disease expressed IFN-γ during a *Leishmania* infection suggests that other factors, possibly IL-4, override the protective effects of IFN-γ. A causal relationship between the effects of IL-4 and susceptibility to infection is suggested by the finding that the inhibition of IL-4 in vivo by monoclonal antibody treatment resulted in the protection of BALB/c mice (35). The association of IL-4 with disease is further supported by earlier results showing that IL-4 was expressed in resistant C57BL/6 mice early in infection, when these mice had lesions, but that IL-4 expression declined as their lesions healed (23, 25). Furthermore, induction of protection in BALB/c mice by a variety of regimens was associated with a decrease in the precursor frequency of IL-4-secreting cells, while the precursor frequency of cells secreting IFN-γ remained unchanged, indicating that the levels of IL-4 were correlated with the outcome of the infection (24). The mechanism of action of IL-4 in a *Leishmania* infection is not known, although some evidence suggests that IL-4 can inhibit macrophage activation, which could result in uncontrolled parasite expansion (16, 17, 38). Therefore, in contrast to the current hypothesis, which proposes that the outcome of a *Leishmania* infection is dependent on the preferential activation of T cells producing either IFN-γ (Th1 cells) or IL-4 (Th2 cells) (10, 37), we propose that IFN-γ is produced by all mice in response to an infection and that it is the dominant effect of IL-4 and/or other concomitantly expressed lymphokines, e.g., IL-10 (11) or transforming growth factor beta (2), that determines the severity of disease.

The spectrum of disease seen for mice infected with *L. major* is similar to that observed for humans infected with different *Leishmania* species. Although the majority of humans infected with parasites causing cutaneous disease show lesion cure, a few develop severe disease. The study presented here was designed as a model for the situation with leishmaniasis patients in which the time of infection and the genetic susceptibility of the host cannot be examined, but the disease severity can be assessed. Interestingly, no correlation was seen between IFN-γ secretion by peripheral blood lymphocytes in response to *Leishmania* antigens in vitro and disease severity in patients with localized lesions (29, 31). These data, like the results presented here, suggest that measuring the level of IFN-γ secreted in response to parasite antigens is not useful in the prognosis of cutaneous leishmaniasis. It may be interesting, therefore, to examine the levels of IL-4 in humans to assess their correlation with the severity of human disease, as suggested by our data obtained with the murine system.

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