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In BALB/c Mice, IL-4 Production During the Initial Phase of Infection with Leishmania major Is Necessary and Sufficient to Instruct Th2 Cell Development Resulting in Progressive Disease

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In contrast to intact BALB/c mice, BALB/c mice rendered deficient in Vβ4+ CD4+ T cells develop a Th1 response to infection with Leishmania major and are resistant. Vβ4-deficient BALB/c mice are unable to generate the early IL-4 transcription occurring in Vβ4 Vo8 CD4+ T cells of BALB/c mice within 1 day of infection. Here we demonstrate that treatment of Vβ4-deficient BALB/c mice with IL-4 during the first 64 h after infection instructs Th2 cell development and susceptibility to infection. The demonstrated inability of IL-4 to reverse the resistant phenotype of BALB/c mice treated with anti-CD4 mAb the day before infection suggest that these effects of IL-4 require its interaction with CD4+ T cells. In contrast to draining lymph node cells from BALB/c mice, cells from Vβ4-deficient BALB/c mice remain responsive to IL-12 following infection. Strikingly, administration of IL-4 to Vβ4-deficient BALB/c mice renders their lymph node cells unresponsive to IL-12 by down-regulating IL-12R β2-chain expression. This study directly demonstrates that in BALB/c mice IL-4 is necessary and sufficient to initiate the molecular events steering Th2 cell maturation and susceptibility to L. major. The Journal of Immunology, 2000, 164: 4819–4825.

Mice from most inbred strains (e.g., C57BL/6, C3H, CBA) are resistant to infection with Leishmania major, obligate intracellular protozoan parasites of mammals in their mammalian hosts. In contrast, mice from most inbred strains (e.g., C57BL/6, C3H, CBA) are resistant to infection with Leishmania major, obligate intracellular protozoan parasites of mammals in their mammalian hosts. In contrast, mice from resistant strains, do not become immune to reinfection. In this model of infection, genetically determined resistance and susceptibility are clearly related to the development of polarized CD4+ Th1 and Th2 responses, respectively (1, 2).

Among the several factors possibly influencing the development of functionally polarized CD4+ Th effector responses, cytokines are the most important stimulus. Results from studies performed in vitro using naïve CD4+ T cells from TCR αβ transgenic mice have clearly implicated IL-12 in the differentiation of Th1 cells (3–5) and IL-4 in the differentiation of Th2 cells (6–8).

The importance of IL-12 for Th1 cell development has been substantiated in vivo using the murine model of infection with L. major (9, 10). Several experimental results also strongly support a requisite role of IL-4 in mediating both Th2 cell differentiation and susceptibility to L. major in BALB/c mice (11, 12). In this context, prior studies from this laboratory have shown that susceptible BALB/c mice, in contrast to mice from resistant strains, exhibited a burst of IL-4 mRNA expression in CD4+ T cells from the draining lymph nodes (LN) within 1 day after infection with L. major (13). This early burst of IL-4 expression occurred in a restricted population of Vβ4-Vo8 CD4+ T cells after cognate recognition of a single epitope of the Leishmania homologue (LACK)* (14) of mammalian RACK1 (15). BALB/c mice rendered deficient in Vβ4+ T cells as a result of neonatal infection with the mouse mammary tumor virus (MMTV(SIM)), encoding a superantigen leading to systemic deletion of CD4+ T cells expressing the Vβ4 TCR chain (16), did not generate early IL-4 transcripts in CD4+ T cells within the first day of infection, developed a Th1 response, and were resistant to infection (15). These results combined with those showing that BALB/c mice tolerant to LACK as a result of the transgenic expression of this molecule in the thymus were resistant to infection (17) clearly indicate that Vβ4-Vo8 CD4+ LACK-reactive cells are required for subsequent Th2 cell development and susceptibility to L. major in BALB/c mice. The critical role of these LACK-reactive cells in the development of aberrant Th2 response in BALB/c mice after infection with L. major was interpreted as a consequence of the IL-4 they rapidly produce during the initial stage of infection. The role of IL-4 in Th2 cell maturation in vivo has also been substantiated in...
other model systems where the supply of exogenous IL-4 at the time of priming in vivo has clearly been shown to induce maturation of specific CD4+ T cells toward the Th2 phenotype (18, 19). Recent results challenged a requisite role of IL-4 in Th2 cell maturation and disease progression by showing that the genetic absence of IL-4 did not modify the susceptible phenotype of BALB/c mice (20). However the possibility exists that in BALB/c mice with a disruption of the IL-4 gene, the critical function of IL-4 in Th2 cell maturation and susceptibility to infection is substituted by an alternative pathway not operational in normal BALB/c mice.

Therefore, we initiated studies to directly assess whether IL-4 alone, during the initial phase of infection, was sufficient to instruct Th2 cell development and susceptibility to L. major in BALB/c mice. In this report we show that treatment of otherwise resistant Vβ4-deficient BALB/c mice with rIL-4 during the first 2.5 days of infection is sufficient to render them fully susceptible to infection.

Materials and Methods

Mice

Female BALB/c, C57BL/6, and B10.D2 mice were purchased from IFFA Credo (St. Germain sur l’Arbresle, France). BALB/c mice infected with either MMTV(SIM) (BALB/c-SIM) or MMTV(SW) (BALB/c-SW), were obtained, as described, from litters nursed within 24 h after birth on MMTV(SIM)- or MMTV(SW)-infected BALB/c mothers, respectively (15). This neonatal exposure to the retrovirus results in a progressive decline in the numbers of designated Vβ9 CD4+ T cells, which is almost completed by 20 wk of age (16, 21). Depletion was confirmed by flow cytometric analysis using the appropriate anti-TCR Vβ-chain mAbs with PBL obtained from mice at the initiation of the experiment. All mice neonatally exposed to MMTV and control mice were used at the age of at least 20 wk.

Parasites and treatment of mice

L. major

LV 39 (MRHO/Sv/59/P strain) were maintained in vivo and in vitro as previously described (22). For infection, mice were infected s.c. in one hind footpad with 10^6 stationary phase L. major promastigotes in a volume of 5 μl. The footpad lesions were measured with a vernier caliper and compared with the thickness of the uninfected footpad. Starting the day of infection with L. major, some mice were injected i.p. eight times with 0.1 μg of murine rIL-4 in PBS-1% normal mouse serum (NMS) every 8 h. Highly purified, murine IL-4 was a gift from Dr. A. Levine (Monsanto, St. Louis, MO) (23). Before infection, the biological activity of the rIL-4 (2 × 10^6 U/mg) was confirmed by a bioassay using the CTLL-44 cell line (gift from P. Erb, University of Basel, Basel, Switzerland) as described previously (24). Recombinant murine IL-4 expressed in X63Ag-653 cells (gift from F. Melchers, Basel Institute of Immunology, Basel, Switzerland) was used as the standard. The limit of detection of the assay was 20 pg/ml.

Statistical analysis

Data were analyzed statistically by Student’s t test for unpaired data.

Results

Administration of exogenous IL-4 during the early stage of infection renders otherwise resistant Vβ4 CD4+ T cell-deficient BALB/c mice fully susceptible to L. major

BALB/c mice rendered Vβ4 CD4+ T cell deficient by prior infection with MMTV(SIM) are incapable of generating the IL-4 transcriptional burst normally occurring in BALB/c mice within the first day of infection with L. major (15). In contrast to control BALB/c or BALB/c mice rendered Vβ6 CD4+ T cell deficient as a result of infection with MMTV(SW), Vβ4-deficient mice subsequently develop a Th1 response in the CD4+ T cell population and express a resistant phenotype (15). Therefore, experiments were designed to determine whether the administration of exogenous IL-4 to otherwise resistant Vβ4-deficient BALB/c mice during the initial period of infection with L. major was capable of reversing their resistant phenotype. The timing of administration of exogenous IL-4 to Vβ4-deficient BALB/c mice used in the present study was chosen based on previous results showing that neutralizing anti-IL-4 Ab was no longer able to redirect Th1 cell development in intact BALB/c mice if administered 72 h after the initiation of infection (26). The results in Fig. 1 confirm that BALB/c mice made Vβ4 CD4+ T cell-deficient (BALB/c-SIM), but not control BALB/c or Vβ6-deficient (BALB/c-SW) mice, were resistant to infection with L. major. Administration of IL-4 only during the first 64 h after infection to Vβ4-deficient BALB/c mice (BALB/c-SIM) fully reversed their resistant phenotype, because these mice developed progressive lesions not substantially different from those in concurrently infected BALB/c or Vβ6 CD4+ T cell-deficient control mice (Fig. 1). Furthermore, the numbers of viable parasites recovered after culture in vitro of footpad tissue under limiting dilution condition (32) confirmed that although parasitic growth was controlled in Vβ4 CD4+ T cell-deficient BALB/c mice compared to that in resistant C57BL/6 mice (~200 parasites/footpad) 6 wk after infection, footpad lesions of Vβ4 CD4+...
response to infection with *L. major* phenotype of Vβ4 only during the initial phase of infection, to reverse the resistant were designed to correlate the ability of exogenous IL-4, given every 8 h during the first 2.5 days of infection. The size of the footpad lesion was monitored using a vernier caliper as described in Materials and Methods. The mean sizes of lesions (five mice per group) and SDs are shown. Similar results were obtained in two other separate experiments.

T cell-deficient BALB/c mice treated with IL-4 during the first 64 h of infection contained numbers of parasites (≈10⁸) similar to those found in control BALB/c mice.

These results demonstrate that IL-4 alone, during the initial phase of infection with *L. major*, was sufficient to allow the uncontrolled development of lesions in otherwise resistant Vβ4-deficient BALB/c mice.

**Treatment with IL-4 during the initial stage of infection with *L. major* redirects Th2 cell development in Vβ4 CD4⁺ T cell-deficient BALB/c mice**

The development of an aberrant polarized Th2 response following infection with *L. major* underlies the exquisite susceptibility of BALB/c mice (2). Prior studies from this laboratory have shown that BALB/c mice rendered Vβ4-deficient developed a Th1 response to infection with *L. major* that closely resembles that of genetically resistant C57BL/6 mice (15). Therefore, experiments were designed to correlate the ability of exogenous IL-4, given only during the initial phase of infection, to reverse the resistant phenotype of Vβ4-deficient BALB/c mice with the ultimate development of polarized Th2 response weeks later.

Together with resistant C57BL/6 mice, groups of BALB/c, BALB/c-SIM, and BALB/c-SW mice were inoculated with *L. major* in the hind footpads. An additional group of BALB/c-SIM mice was treated with rIL-4 during the initial 2.5 days of infection. After 6 wk, a time when polarized Th differentiation can be demonstrated, mice were sacrificed, and their draining LN were harvested for cytokine mRNA and protein assays. Results in Fig. 2 confirm that Vβ4-deficient BALB/c-SIM mice developed a Th1 response similar to that seen in genetically resistant C57BL/6 mice, with almost undetectable levels of IL-4 transcripts. In sharp contrast, compared with uninfected control mice, BALB/c and Vβ6-deficient BALB/c-SW mice had an important increase in the amounts of IL-4 transcripts. Remarkably, Vβ4-deficient BALB/c-SIM mice treated with IL-4 during the initial phase of infection with *L. major* had increased amounts of IL-4 transcripts comparable to those occurring spontaneously in similarly infected BALB/c or Vβ6-deficient BALB/c-SW mice (Fig. 2). It is noteworthy that the IL-4 mRNA observed in BALB/c-SIM mice treated with rIL-4 during the initial 2.5 days of infection with *L. major* was produced by CD4⁺ T cells expressing a wide range of Vβ TCR chains (results not shown). Comparable results were observed when the supernatants of cultures of specifically stimulated, designated LN cell populations were analyzed for the accumulation of IL-4 (Fig. 2). As previously observed, it is noteworthy that the responses of the various groups of mice could not be discriminated on the basis of either the amounts of IFN-γ transcripts or the IFN-γ produced in vitro. Analogous results were observed using purified CD4⁺ T cell populations for cytokine mRNA assays (data not shown). Thus, as assessed by analysis of both mRNA and protein production, administration of IL-4 to Vβ4-deficient BALB/c SIM mice during the initial 2.5 days of infection with *L. major* is sufficient to instruct the development of an IL-4-producing Th2 response.
Numerous interventions have been shown to render otherwise susceptible BALB/c mice resistant to infection with *L. major*. Among these, treatment with depleting anti-CD4 mAbs at the initiation of infection has clearly been shown to result in Th1 cell development, inhibition of Th2 cell maturation, and resistance to infection has clearly been shown to result in Th1 cell maturation, and resistance to infection with *L. major* stationary phase promastigotes in one hind footpad, the course of infection was monitored in BALB/c mice and BALB/c mice injected 48 h prior to infection with 600 µg of anti-CD4 mAb GK 1.5 and treated or not with the total of 0.8 µg of IL-4 during the first 2.5 days of infection (five mice per group). The mean size of lesions and SDs are shown. Similar results were obtained in three individual experiments.

FIGURE 3. Administration of IL-4 during the first 64 h of infection with *L. major* to CD4-depleted BALB/c mice does not result in Th2 cell maturation. Six weeks after infection draining LN cells from the mice designated in Fig. 3 were specifically stimulated in vitro with UV-irradiated *L. major*. After 72 h of culture IL-4 and IFN-γ production in supernatants was measured as described in Materials and Methods. For each determination background levels of cytokines in supernatants of cultures without *L. major* were subtracted. Bars represent the mean and SD of triplicate determinations. Statistical significance for cultures of draining LN cells from anti-IL-4 mAb-treated BALB/c mice vs cultures of cells from control BALB/c mice is indicated as follows: NS, not significant; *, p < 0.05. Similar results were obtained in two separate experiments.

**Discussion**

These data establish that IL-4, given only during the first 2.5 days after infection with *L. major*, by itself is sufficient to redirect Th2 cell development and susceptibility to *L. major* in BALB/c mice expression and, conversely, 2) the effect of treatment with exogenous IL-4 during the early stage of infection on redirecting Th2 cell development and susceptibility of these mice to infection with the loss of responsiveness to IL-12 and down-regulation of the IL-12R β2-chain expression.

Groups of BALB/c, Vβ6-deficient, and Vβ4-deficient BALB/c mice were inoculated in the hind footpads with *L. major* promastigotes. One additional group of Vβ4-deficient BALB/c mice was also treated with a total of 0.8 µg of IL-4 during the first 64 h after the inoculation of parasites. Five days later, draining LN cells were stimulated with *L. major* in vitro in the presence or the absence of exogenous IL-12, and the levels of IFN-γ were determined in supernatants after 3 days of culture. In parallel, analysis of IL-12R β1- and β2-chain mRNA expression in freshly harvested LN cells was performed using RT-PCR. The results in Fig. 5 show that specifically stimulated LN cells from either BALB/c or Vβ6-deficient BALB/c-SW mice do not respond to IL-12 in terms of enhanced IFN-γ production in vitro. RT-PCR analysis also revealed that the IL-12R β2-chain mRNA was absent in LN cells from these mice (Fig. 6). In sharp contrast, similar analysis in Vβ4-deficient BALB/c-SIM mice showed that LN cells were responding to IL-12 and expressed IL-12R β2-chain mRNA at high levels (Figs. 5 and 6). Strikingly, treatment of Vβ4-deficient BALB/c-SIM mice with IL-4 during the first 2.5 days of infection resulted in a loss of LN cell responsiveness to IL-12 and IL-12R β2-chain mRNA expression. Comparable results were obtained with LN cells harvested 8 days after infection (data not shown).

Together, these results demonstrate a stringent correlation between, on the one hand, the absence of IL-4 during the early stage of infection, the maintenance of IL-12 responsiveness, and the development of a protective Th1 response and, on the other hand, the presence of IL-4, the loss of responsiveness to IL-12, and the development of a disease-promoting Th2 response.

**FIGURE 4.** Administration of IL-4 during the initial phase of infection with *L. major* to CD4-depleted BALB/c mice does not result in Th2 cell maturation. Six weeks after infection draining LN cells from the mice designated in Fig. 3 were specifically stimulated in vitro with UV-irradiated *L. major*. After 72 h of culture IL-4 and IFN-γ production in supernatants was measured as described in Materials and Methods. For each determination background levels of cytokines in supernatants of cultures without *L. major* were subtracted. Bars represent the mean and SD of triplicate determinations. Statistical significance for cultures of draining LN cells from anti-IL-4 mAb-treated BALB/c mice vs cultures of cells from control BALB/c mice is indicated as follows: NS, not significant; *, p < 0.05. Similar results were obtained in two separate experiments.
the responsiveness to IL-12 in vitro of draining LN cells isolated 5 days after parasite inoculation. BALB/c and BALB/c mice deficient in either Vβ4+ (BALB/c-SIM) or Vβ8+ CD4 T cells (BALB/c-SW) were inoculated in one hind footpad with 3 × 10^6 stationary phase L. major promastigotes. Vβ4-deficient BALB/c mice were also treated with either 0.1 µg of IL-4 in PBS-1% NMS or PBS-1% NMS alone i.p. every 8 h during the first 2.5 days of infection. Five days after infection draining LN cells were specifically stimulated with UV-irradiated L. major in the absence or the presence of exogenous IL-12 (10 ng/ml). After 72 h IFN-γ in culture supernatants was measured as described in Materials and Methods. For each determination, background levels of IFN-γ in cultures without L. major were subtracted. Bars represent the mean and SD of triplicate determinations. Statistical significance for cultures containing IL-12 vs control cultures is indicated as follows: NS, not significant; *, p < 0.05. Similar results were obtained in two separate experiments.

rendered resistant as a result of deletion of Vβ4+ CD4+ T cells. These Vβ4-deficient BALB/c mice have been shown to be incapable of generating the early IL-4 transcriptional burst normally occurring in BALB/c mice among Vβ4 Vα8 CD4+ LN cells within the first day after the s.c. inoculation of parasites (15). The use of Vβ4-deficient BALB/c mice, besides having made possible the present demonstration of the essential role of IL-4 during the early stage of infection in instructing Th2 cell development and susceptibility to L. major in BALB/c mice, should also permit us to define the boundaries in time that limit this effect of IL-4. Experiments are currently in progress to define the window of time after infection during which exogenous IL-4 redirects Th2 cell development in Vβ4-deficient BALB/c mice. The results presented in this paper, obtained using Vβ4-deficient BALB/c mice, complement those showing that treatment with neutralizing anti-IL-4 Abs was capable of redirecting Th1 cell development in intact BALB/c mice (12). In this context, we have pinpointed a short time, between 16 and 48 h after infection, during which IL-4 must be present to instruct susceptibility through Th2 cell development in intact BALB/c mice, because neutralization of IL-4 after that time no longer impeded Th2 cell maturation (26).

The rapid IL-4 response to the parasite of Vβ4 Vα8 CD4+ BALB/c cells is focused on a single dominant I-Aβ-restricted T cell epitope of the LACK Ag of L. major (15, 35). The present demonstration that exogenous IL-4, only transiently supplied at the initiation of infection, is able of acting in Vβ4-deficient BALB/c mice as a substitute for the Vβ4 Vα8 CD4+ T cells reactive to the LACK I-Aβ epitope implies that an essential role of these cells is to provide the IL-4 necessary for Th2 cell maturation. This conclusion is supported by prior results showing that the specific induction of an unresponsive state in the LACK I-Aβ epitope-reactive Vβ4 Vα8 CD4+ T cells following treatment of BALB/c mice with altered LACK proteins that differ by a single amino acid from the natural I-Aβ-restricted epitope was capable of 1) antagonizing the early IL-4 burst in response to LACK and 2) redirecting the otherwise ineffective Th2 response to a fully protective Th1 response with long term protection (35). Thus, in the absence of the IL-4 produced during the early stage of infection by LACK-reactive Vβ4 Vα8 CD4+ T cells, Th cell development defaults to the Th1 pathway, with the resulting establishment of protective immunity to this otherwise lethal infection.

Observations showing that deletion of LACK-reactive Vβ4 Vα8 CD4+ T cells, either through superantigen (MMTV(SIM))-mediated deletion of Vβ4-expressing CD4+ T cells (15) or by thymic expression of LACK as a transgene (17), led to a resistant phenotype in BALB/c mice already strongly supported the essential role of these cells in susceptibility to infection with L. major. These cells have also been shown to expand in the draining LN as late as 3 wk after infection (36). The results of the present study, showing that their absence can be fully substituted by exogenous IL-4 given only during the early stage of infection, further suggest that their role in the development of progressive disease might be limited to the production of the IL-4 necessary for Th2 cell development and that they are not essential at the effector stage of the Th2 response. This contention is also supported by our recent observation that infection of BALB/c mice with MMTV(SIM) 3 days after parasite inoculation, i.e., a time when the early burst of IL-4 mRNA expression had occurred, neither modified their susceptible phenotype nor inhibited the magnitude of the Th2 response in their draining LN despite drastically reduced numbers of Vβ4 CD4+ T cells as tested in their blood and draining LN (H. Himmelrich, unpublished observations).

Similarly to Vβ4-deficient BALB/c mice, BALB/c mice transiently depleted of CD4+ T cells by treatment with anti-CD4 mAb GK 1.5 the day before infection mount a Th1 response and are resistant to L. major. The early burst of IL-4 mRNA expression normally seen in the draining LN of BALB/c mice in response to infection was also not induced in CD4+ T cell-depleted BALB/c mice (13). However, early IL-4 mRNA expression, Th2 cell development, and progressive disease did occur when parasites were injected 25–30 days after anti-CD4 mAb treatment at a time that coincides with repopulation of lymphoid organs with a significantly sized pool of CD4+ T cells (13) containing sufficient numbers of Vβ4 Vα8 CD4+ T cells rapidly producing IL-4 in response to the LACK Ag of L. major (P. Launois, unpublished observations). Thus, in the absence of IL-4 differentiation of the CD4+ T
cell precursors with specificities for several parasite-derived epitopes gradually repopulating the lymphoid tissues of BALB/c mice treated with anti-CD4 mAb defaults to the Th1 pathway, leading to resistance to infection. In contrast to its unambiguous effect in Vβ4-deficient BALB/c mice, treatment with exogenous IL-4 during the early phase of infection induced neither Th2 cell development nor susceptibility to L. major in otherwise resistant anti-CD4 mAb-treated BALB/c mice. Inasmuch as the exogenous IL-4 was given during the first 2.5 days of infection, a time of maximal depletion of CD4⁺ T cells in anti-CD4 mAb-treated mice and given the short half-life of IL-4 in vivo, these results strongly suggest that these effects of IL-4 require its direct interaction with parasite-specific CD4⁺ T cell precursors.

Previous results have documented, on the one hand, the rapid induction of a state of unresponsiveness to IL-12 in CD4⁺ T cells from susceptible BALB/c mice and, on the other hand, the maintenance of responsiveness to IL-12 in CD4⁺ T cells from resistant C57BL/6 mice (26). The extinction in BALB/c mice and the maintenance in C57BL/6 mice of IL-12 signaling correlated with down-regulation and maintenance of IL-12R β2-chain mRNA expression, respectively (28, 37). This demonstration in vivo of the importance of IL-12R β2 subunit expression for IL-12 signaling confirmed results obtained in vitro using TCR αβ transgenic CD4⁺ T cells (38). The results in this report showing that in contrast to wild-type BALB/c mice, LN cells from Vβ4-deficient BALB/c mice maintain IL-12R β2 subunit expression and their capacity to respond to IL-12 in vitro in terms of IFN-γ production support the concept that in vivo loss or maintenance of IL-12 signaling is an important step in the stable commitment of naive CD4⁺ T cell precursors to the Th2 or Th1 pathway of maturation, respectively (28). Importantly, the presently demonstrated capacity of exogenous IL-4 to interfere with maintenance of IL-12R β2-chain expression and IL-12 signaling in LN cells of Vβ4-deficient BALB/c mice provides definite evidence for the essential role of the IL-4 rapidly produced in intact BALB/c mice by Vβ4 Voβ CD4⁺ T cells in the induction of a state of unresponsiveness to IL-12. These data provide direct support for the view that overproduction of IL-4 in the early stage of infection with L. major, secondarily inducing extinction of IL-12 signaling, underlies the genetic tendency of BALB/c mice to mount an aberrant Th2 response to L. major.

It is worth mentioning that administration of exogenous IL-4 to C57BL/6 mice at the dose used in this study resulted in neither modification of their resistant phenotype nor redirection of the effector cells to the Th2 phenotype. In addition, similarly to control infected C57BL/6 mice, LN cells from Vβ4-deficient BALB/c mice treated with IL-4 during the early stage of infection maintained IL-12R β2-chain mRNA expression and responsiveness to IL-12 in vitro at least up to 8 days after infection (data not shown). These results probably do not stem from differences in sensitivity to IL-4 between CD4⁺ T cells from susceptible and resistant mice, since it has been previously reported that administration of extremely high amounts of IL-4 to C3H mice did not reverse their resistant phenotype (11). IFN-γ has been shown to override the IL-4-induced down-regulation of IL-12R β2-chain mRNA expression both in vitro (38) and in vivo (28). It is thus likely that either CD4⁺ T cells from C57BL/6 mice are more sensitive to IFN-γ than BALB/c CD4⁺ T cells or the levels of endogenous IFN-γ or that rapidly produced in response to infection with L. major are higher in C57BL/6 than in BALB/c mice. The first hypothesis might also be the basis for the results of elegant experiments using TCR αβ transgenic CD4⁺ T cells that have shown that B10.D2 cells retained IL-12 responsiveness after specific activation in vitro even in the presence of an excess of BALB/c T cells (39) and the IL-4 they produce.

Results showing that genetically pure BALB/c mice deficient in IL-4 neither resolved their cutaneous lesions nor redirected their CD4⁺ T cell response toward the Th1 phenotype (20) have recently disputed the essential role of IL-4 in susceptibility to L. major and Th2 cell maturation. However, independent studies also performed with IL-4 knockout BALB/c mice have generated conflicting results (40). These differences, although still largely unexplained, were recently attributed at least in part to the use of different strains of parasites (41). Recent extension of these investigations using both IL-4-deficient and IL-4R α-chain-deficient BALB/c mice did not permit this controversy to be settled. In addition to confirming the previously reported conflicting results obtained using IL-4-deficient BALB/c mice, the data obtained using IL-4R α-deficient BALB/c mice suggested, on the one hand, that IL-13, signaling through the IL-4R α-chain, might be involved in disease progression (41) and, on the other hand, that IL-13 could be involved in controlling dissemination of parasites during the late and chronic phase of infection (42). Nevertheless, confronted with the present observation directly showing the requisite role of IL-4 during the early phase of infection for Th2 cell maturation leading to progressive disease in genetically intact BALB/c mice, an unimpaired Th2 cell maturation and susceptibility to L. major in IL-4 knockout BALB/c mice could suggest the induction in these mice of a compensatory factor(s) able to replace the normally essential function of IL-4. Such a factor(s), able to substitute IL-4 in IL-4-deficient mice, probably also originates from CD4⁺ T cells, because IL-4 knockout BALB/c mice transiently depleted of CD4⁺ T cells at the beginning of infection controlled parasite growth (43).

In summary, this study directly indicates that IL-4 during the initial period of infection with L. major is necessary and sufficient to set in motion the molecular events, including down-regulation of the IL-12R β2 expression in CD4⁺ T cells, ultimately resulting in Th2 cell maturation and susceptibility to infection in BALB/c mice. These results give further strength to the data that have implicated LACK-reactive Vβ4 Voβ CD4⁺ T cells as initiators of the events ultimately mediating the susceptible phenotype of BALB/c mice infected with L. major. Further analysis of the molecular basis for their rapid activation and IL-4 production following parasite inoculation will significantly contribute to understanding of the mechanism(s) underlying Th2 cell development in this model system.

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