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Factores inmunológicos que gobiernan la resistencia y susceptibilidad del ratón a la infección por *Leishmania major*

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REVIEW ARTICLE

Immunological factors governing resistance and susceptibility of mice to *Leishmania major* infection

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ABSTRACT. Infection with *Leishmania* sp. is particularly suitable for the study of immunoregulatory mechanisms associated with host susceptibility or resistance. The clinical spectrum of this infection results from parasite virulence factors and host immune responses, some of which acting in a host protective manner while others exacerbate the disease. In the mouse model, factors governing resistance to *Leishmania major* infection mainly depends on the IFN-γ activation of the leishmanicidal function of macrophages, and the Fas/FasL-dependent T-cell cytotoxicity against infected macrophages. On the other hand, the immunological factors of susceptibility involve: I) the early upregulation of IL-4 production induced by the LACK antigen, II) the upregulation of IL-2 production, III) the high production of TGF-β as macrophage deactivating factor, and IV) the production of IL-10 by the *L. major* infected macrophages, inhibited their microbicidal activity.

Key words: *Leishmania* / Immune Response / Th1/Th2 Paradigm / susceptibility to *Leishmania*.

RESUMEN. La infección con *Leishmania* sp. es particularmente adecuada para el estudio de mecanismos inmunorregulatorios asociados con la susceptibilidad o resistencia del huésped. El espectro clínico de esta infección es el resultado de los factores de virulencia del parásito y las respuestas inmunes del huésped, algunas de las cuales actúan en la protección del huésped, mientras que otras exacerban la enfermedad. En el modelo de ratón, los factores que gobiernan la resistencia a la infección a *Leishmania major* principalmente depende de la activación de IFN-γ de la función leishmanicida de macrófagos, y la citotoxicidad de células T dependiente de Fas/FasL contra macrófagos infectados. Por otro lado, los factores inmunológicos de susceptibilidad involucran: I) La regulación positiva temprana de la producción de IL-4 inducida por el antígeno LACK, II) La regulación positiva de la producción de IL-2, III) La elevada producción de TGB-β como factor desactivante de macrófagos, y IV) La producción de IL-10 por los macrófagos infectados con *L. major*, inhibida su actividad microbicida.

Palabras clave: *Leishmania*, respuesta inmune, paradigma/Th1/Th2/, susceptibilidad a *Leishmania*.

1. *LEISHMANIA* AND LEISHMANIASIS

Leishmania parasites include different complexes of species transmitted by blood suckling sandflies. The infected vectors inoculate the promastigote form of the parasite (with external flagellum) in the dermis of a vertebrate host (man, dog and numerous other mammals). Some promastigotes attach to surface receptors of dermal macrophages and are *phagocytosed*. Within the macrophage parasitophorous vacuoles, where they resist destruction in spite of phagosome fusion with the lysosomes, the promastigotes rapidly transform into amastigotes (spherical organisms with no external flagellum) and multiply, amastigote-laden macrophages burst and the parasites reinfect others cells. The cutaneous Langherans cells (skin dendritic cells) may get infected by *Leishmania* and harbour parasites for prolonged periods of time.[1] These cells are the main antigen-presenting cells (APC) of the skin, which, after priming, migrate to the regional lymph node where they stimulate T cells.[2-6]

Leishmaniasis belong to the group of the six most important tropical diseases in the world recognized by the WHO/TDR. Twelve to 15 million people are infected in 88 countries, with 3-4 million suffering from the disease. The population at risk of infection is estimated to be 370 millions. Leishmaniasis occurs in three major clinical forms: visceral, cutaneous and mucocutaneous leishmaniasis.

Cutaneous leishmaniasis is the most widespread form of the disease, and it is found in Africa, India, Latin America, South West Asia and the Mediterranean basin. The clinical spectrum of the disease includes immune responder individuals with the localized cutaneous form of the disease (LCL) and non-responder individuals with disseminated or diffuse cutaneous lesions (DCL), which occur less frequently. The phylogenetic complexes *L. major* and both *L. mexicana* (syn: *L. pifanoi*) and *L. amazonensis* (syn: *L. garnhami*), produce such clinical forms in the Old and the New world, respectively.[7,8]

2. THE CONCEPT OF SUSCEPTIBLE AND RESISTANT MICE: THE PARADIGM OF EXPERIMENTAL LEISHMANIASIS

Leishmaniasis in mice is an excellent model to study the extremes of host-parasite relationship. Indeed, while the genetic definition of mouse strains progressed, two clearly distinct phenotypes of leishmaniasis could be observed depending on the mouse strain used: resistant mice (C57BL/6, CBA, C3H, C3H/He) develop cutaneous lesions that re-

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solve spontaneously, whereas susceptible mice (BALB/c, DBA, A/Jax) allow dissemination of the infection from their non-healing primary cutaneous lesions.[9]

This dichotomy has allowed identification of murine genes involved in the outcome of infection. The *Nramp1* gene (essential for *L. donovani* and *L. mexicana*, but less for *L. major*), encodes a protein with transporter function in macrophages.[10-12] The H-2-linked gene (RID-I), mapped in the I-E and I-A regions of the murine MHC (chromosome 17) is involved in the control of *L. donovani, L. mexicana* and *L. major* infections.[13,14] *H-2* genes are also associated with the visceral and metastatic spread of *L. mexicana*-infection.[15] In contrast, *L. major*-, but not *L. donovani*- or *L. mexicana*, infection is controlled by two autosomal loci: *Imr1* at the H-2 locus, and *Imr2* on chromosome 9. Another locus, *lmr3*, lying on the X chromosome, whose effect depends on a specific *lmr1* haplotype, has also been identified.[16,17] The *Scl1* gene, on chromosome 11, encodes a protein affecting the macrophage processing and the presentation of *L. major* antigens.[18-20] The *Scl2* gene (on chromosome 4) is associated with the resistant phenotype of DBA/2 mice to infection with *L. mexicana*.[21,22] Since, this resistance is mainly found in females, it appears to operate on macrophage activation through an oestrogen receptor.[23,24] The *Ir-2* linked gene, on chromosome 2, controls infection by *L. donovani*.[25,26] The *H-11* gene, still unmapped, affects mainly the susceptibility to *L. major* and would exert its influence by regulating MHC gene expression.[15,27]

Such clear-cut differences in response to *Leishmania* infections among mouse strains are of crucial interest for immunologists. The Th1/Th2 concept, originally established on the basis of different cytokines produced by T-cell clones,[28,29] has become of prime importance for the understanding of heterogeneous responses of the immune system and their implications for infectious diseases.[30-32] It has been legitimated first in the mouse model of cutaneous *L. major* infection,[30-32] and experimental leishmaniases is always the subject of fundamental immunological studies.[33] Although the relationship between the immunological factors and the genes involved in susceptibility and resistance to infection has not still been clarified, it is interesting to note that the genetic region containing the loci for susceptibility to *L. major* (*Scl1*), also contains the genes for IL-12p40, iNOS, IL-4 and IL-10, as well as those for chemokines.[20,34]

3. IMMUNOLOGICAL FACTORS GOVERNING RESISTANCE TO *L. MAJOR* INFECTION

The control of *L. major* infection in resistant mice (C57BL/6) depends on the activation of two main immunological mechanisms:

The IFN-γ activation of leishmanicidal function of macrophages

Production of nitric oxide (NO) mainly mediates destruction of intracellular *Leishmania* amastigotes by infected and activated macrophages.[35] This has been clearly shown in *in vitro* and *in vivo* experiments by using NO inhibitors,[36-38] or by disruption of the NOS2 gene in resistant mice, this abrogating their ability to control the infection.[39]

IFN-γ is clearly a key cytokine eliciting the leishmanicidal function of host macrophages.[36,40,41] Resistant mice with targeted disruption of the genes encoding for IFN-γ, IFN-γ receptor, or IRF1 (a transcription factor for IFN-γ) are unable to restrict the growth of *L. major* and suffer from fatal infection.[42-45]

In vitro IFN-γ can activate macrophages alone or in synergy with TNF-α[46-48] or IL-7.[49] However, it is now clear that NO can be produced *in vivo* in a TNF-independent manner, as shown by infection of mice lacking the TNF receptor p55 (TNFR1), which make NO and kill *Leishmania* parasites.[50] The alternative second signal for macrophage activation in these animals might derive from the CD40/CD40L interaction, and/or parasite immunostimulatory molecules analogous to lipopolysaccharide (LPS).

IFN-γ is produced by CD4 Th1 cells during the course of *L. major* infection.[51] Co-stimulation by CD28/CD80, (B7-1) rather than CD86 (B7-2), and CD28/CD152 (CTLA4) is required for CD4 activation, amplification of IFN-γ production and resolution of *L. major* infection.[52,53] The participation of NK cells to IFN-γ production (innate immunity) remains controversial, since contradictory results have been reported on infection of resistant mice with deficient NK functions.[45,54-56]

IL-12, a well known NK-activating cytokine and inducers of IFN-γ production by Th1 cells plays a key role in the control of *L. major* infection. Indeed, administration of anti-IL-12 antibody in C57BL/6 mice abrogates the control of the lesion[57] and 129Sv/Ev or C57BL/6 mice lacking IL-12 p35 or p40, develop large and progressive lesions similar to those of BALB/c mice when infected with *L. major*.[58,59]

The effect of *Leishmania* on *in vitro* production of IL-12 by macrophages depends on the parasite stage and form. Procyclic promastigotes and amastigotes induce IL-12 production,[60,61] as well as the *L. major* ribosomal protein LeIF.[62-64] By contrast, the metacyclic promastigotes strongly inhibit IL-12 production.[65] Downregulation of IL-12 production occurs after the parasite adhesion to macrophage receptors, particularly to the complement receptor 3 (CR3),[66,67] a potential receptor for the entry of *Leishmania* in macrophages. Indeed, dendritic cells (DC), but not macrophages, have been shown to be the main *in vivo* source of IL-12 following *L. major* infection, although the timing of IL-12 production is still a mat-

ter of debate.[60,68-70] IL-12 production in *L. major* infection is also triggered through a T-cell-dependent pathway, by the interaction of CD40 on IL-12 producing cells, with CD40 ligand (CD40L) on activated CD4 T-cells. This has been shown by administration of anti-CD40 mAb into *L. major*-infected mice[71] or by infection of mice bearing a mutation in the CD40 or CD40L genes.[72-74] Interestingly, recent data indicate that NOS2 activity is necessary for IL-12 signaling through its central signal transducer Stat4 to prevent the spreading of *L. major* infection in mice.[75]

The Fas/FasL-dependent T-cell cytotoxicity against infected macrophages

Activated CD4+ Th1 cells expressing FasL (CD95L, Apo1L) on their surface are able to induce the apoptotic death of *L. major*-infected macrophages expressing Fas antigen on their membrane when activated with IFN-γ.[76,77] The *in vivo* relevance of such a mechanism was shown by using C57BL/6 mice deficient in Fas antigen (*lpr* mutation) or FasL (*gld* mutation), which were unable to resolve the lesions induced by *L. major*, although they mounted a CD4+ Th1 response with macrophages producing NO.[77,78] However, the Fas/FasL-dependent protective response appears late in infection (only 6 weeks after parasite inoculation), and although lesions in mutant mice display a non-healing phenotype, lesion sizes never reach those seen in susceptible mice. Collectively, these data indicate that the Fas-FasL pathway is complementary to the CD40L/IL-12/IFN-γ/NOS2-dependent early mechanism to control the parasite initial replication.

Other factors, such as certain chemokines, are also likely involved in murine resistance to leishmaniasis,[79] but their role in the cascade of events controlling infection is not still clear. Interestingly, although previously suggested,[80,81] the MHC class I-dependent CD8 cytotoxicity fails to control the primary cutaneous *L. major* lesion, as shown by experiments with C57BL/6 mice deficient in the β2mG- or perforin-genes.[7,82,83] This, likely, relies on the particular pathway of *Leishmania* parasites in the parasitophorous vacuole within macrophages, leading to a preferential association to MHC class II molecules for antigen presentation.[84] The intracellular habitat of *Leishmania* also makes antibodies irrelevant in protection.[85,86]

4. IMMUNOLOGICAL FACTORS INVOLVED IN THE SUSCEPTIBILITY TO *L. MAJOR* INFECTION

Upregulation of IL-4 production and LACK antigen

Administration of anti-IL-4 mAb[87,88] or recombinant soluble IL-4 receptor[89] into BALB/c mice rendered them resistant to infection by *L. major*. Forced expression of IL-4 using transgenic constructs interfered with the clearance of parasites in mice on the 129/Sv background[90,91]

Other studies converge to indicate IL-4 as a key cytokine involved in murine susceptibility to *L. major* infection, since its early production, 16 to 48 h after parasite inoculation, results in the further development of a Th2 response, blocking the IFN-γ-dependent parasiticidal effect.[92] The effect of IL-4 in *L. major* infection relates to the downregulation of the β2 chain of IL-12R and to the subsequent IL-12 unresponsiveness.[93] Interestingly, a locus named *Tpm1* (T-cell modifier phenotype-1) controlling the unresponsiveness to IL-12 has been mapped in the murine chromosome 11, that also contains the genes for IL-4, IL-13 and *Scl1* involved in murine susceptibility to *L. major*.[20,94-96]

IL-4 is not released by BALB/c CD4+ NK1.1+ cells,[97-99] but it is by conventional I-A^d class II-restricted CD4+ T cells expressing a Vβ4 Vα8 TCR.[92] The latter cells recognize a single epitope (aa 156-173) on an antigen of *L. major*, called LACK (*Leishmania* homologue receptor for Activated C-Kinase), because of its homology with the mammalian receptors for activated protein kinase C. LACK antigen accounts for about 0.05 % of total protein in *Leishmania* promastigotes.[51,92,100]

An elegant experiment showed that transgenic BALB/c mice expressing the LACK antigen in the thymus were tolerant to this antigen and resistant to infection with *L. major*.[101] Another demonstration corroborating the requirement for LACK recognition in the susceptibility of BALB/c mice, involved the deletion of CD4 T-cells that expressed the Vβ4 TCR by a superantigen encoded by mouse mammary tumor viruses (MMTVs).[92,102,103] It has been shown that the LACK antigen is cross-reactive with peptides derived from microbial antigens of the intestinal flora.[104] So, infection with *L. major* could stimulate LACK-like-memory T-cells to produce more rapidly IL-4. Interestingly, we have recently shown that the T-cell responses to the immunodominant LACK antigen do not play a critical role in determining susceptibility of BALB/c mice to *L. mexicana*.[105] This indicates that the mechanisms responsible for susceptibility to *L. major* are not necessarily valid for all *Leishmania* species.

Contradictory results have been published using IL-4 or IL-4Rα gene deficient BALB/c mice,44,106-108 whereas STAT6 (involved in IL-4 signaling) gene deficient mice became resistant to *L. major* infection compared to wild type animals.109 This suggested the possible involvement of IL-13, a cytokine closely related to IL-4, and sharing receptor components, the STAT6 signaling pathway and biological functions.[110-112]

Indeed, the role of IL-13, in association with IL-4, in the susceptibility to *L. major* infection has been clearly established in experiments with transgenic C57BL/6 mice over

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expressing IL-13 and BALB/c animals deficient in IL-13 and/or IL-4 expression.[113]

Upregulation of IL-2 production and disease exacerbation

IL-2 is abundantly produced in lymph nodes, but not in the spleen of BALB/c mice infected with *L. major*.[114] Treatment of such mice with either anti-IL-2 or anti-IL-2R mAbs leads to the healing of the lesions.[115] The mechanism by which IL-2 exacerbates disease remains unclear. A first possible mechanism relates to the requirement of activated Th2 CD4+ cells by IL-2 to produce IL-4,[115,116] while IFN-γ can be produced by NK cells activated by IL-12, in the absence of IL-2.[117] Second, IL-2 might activate a particular CD4 T-cell population expressing the IL-2R α chain (CD25) characterized by its strong capacity to expand and to produce IL-10, an antagonistic factor of the Th1 immune response;118 whether the role of IL-10 production seems limited as a factor of susceptibility in L. major infection, its role might be important in infections with other *Leishmania* species. Third, IL-2 might also suppress the parasite intracellular killing by activated macrophages through the secretion of TGF-β (see below), but the reported results are contradictory.[119,120] Fourth, a direct effect of IL-2 promoting parasite replication, as observed both in promastigote cultures and after administration of rIL-2 in the lesions of *L. mexicana* and *L. amazonensis*-infected mice cannot be excluded, although a parasite receptor for IL-2 has not been identified.[121,122]

High production of TGF-β as a macrophage deactivating factor

In vitro infection of murine peritoneal macrophages with *L. amazonensis* leads to TGF-β production, and addition of exogenous TGF-β leads to increased cell infection.[123] Susceptible mice infected with *L. major* displayed considerably more cutaneous TGF-β+ cells than resistant animals, as soon as 8 days after infection. Their TGF-β levels inversely correlated with the levels of NOS2 within the lesions.[37] Treatment with TGF-β clearly promotes disease in resistant mice infected with *L. amazonensis*, *L. braziliensis* or *L. major*, while treatment of susceptible BALB/c mice with anti-TGF-β Ab promotes both resistance to *L. amazonensis* and the development of a more pronounced Th1-type response.[123,124] Indeed, TGF-β exerts potent inhibitory effects on macrophage functions, including IL-12 production,[125,126] induction of NOS2 and generation of anti-parasite reactive nitrogen intermediates.[40,127,128]

IL-10 production and susceptibility to leishmaniasis

IL-10 is detected in cells of susceptible mice[129] and *L. major*-infected macrophages are able to produce IL-10 and to inhibit their antimicrobial activity *in vitro*.[130] The *in vivo* administration of rIL-10 alone or associated to IL-4 inhibits the IFN-γ production in *L. major*-infected resistant mice.[88,131,132] However, administration of anti-IL-10 mAb at the time of infection in susceptible mice does not alter the disease progression.[131,133] This suggests that IL-10, known to inhibit IFN-γ- and IL-7-induced activation of macrophages, IL-12 production and the Th1 cell development, does not likely play a significant role in murine susceptibility to *L. major* infection. By contrast, recent unpublished data of our laboratory indicate that IL-10 might have a more important role in the susceptibility to *L. mexicana* infection (Aguilar-Torrentera et al., in preparation).

In conclusion, infection with *L. major* has been a particularly fecund paradigm for geneticists and immunologists. It has allowed the discovery of new genes, immunological mechanisms and to base the concept of a dichotomy between antagonistic immune responses, an essential principle for our understanding of infections diseases. However, for microbiologists, it appears now clearly that the mechanisms involved in susceptibility and resistance to *L. major* infection cannot be extrapolated to other *Leishmania* species, for which such mechanisms are likely to be different.

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