Experimental Murine Leishmaniasis and the Th1/Th2 Cell Concept

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Herein, the current knowledge about the immune response during murine cutaneous leishmaniasis is summarized. Special regard is given to the characteristics and generation of Th1 and Th2 cells during this disease. Originally established on the basis of different cytokines produced by T cell clones, it is now known that the Th1/Th2 concept really defines totally different immune pathways that affect most, if not all cells of the immune system. Murine experimental leishmaniasis was the first model to confirm the relevance of the Th1/Th2 concept *in vivo*. In particular, data from this laboratory will be presented on the role of different IL-4 receptor allotypes, on the role of the transcription factor interferon-regulatory-factor-1 (IRF-1) and the importance of the enzyme inducible NO synthase (iNOS).

The model of experimental cutaneous leishmaniosis

Protozoan parasites of the genus *Leishmania* are transmitted by sandflies and exist in two forms. Flagellated, promastigote forms live in the gut of the sandfly. When the sandfly bites a host, these forms are transmitted into the skin, where they enter macrophages. Here, they transform into an amastigote form that loses the flagellum and multiplies inside the macrophage. After disruption of the macrophage, more macrophages become infected. Cell types other than macrophages, e.g. epidermal Langerhans cells [1] and fibroblasts (C. Bogdan, unpublished observation), may also become infected. Depending on the species of *Leishmania*, the developing disease is either harmless and self-healing, e.g. during infections with *L. major*, or systemic and fatal (e.g. *L. donovani*). All *Leishmania* species infect various animals (e.g. rodents and dogs) in addition to humans.

The infection of mice with *L. major* has been used as a model to analyze the parameters of the immune system that are responsible for cure or death of the infected host. Depending on the inbred mouse strain used, this infection is either fatal (e.g. in susceptible BALB/c mice) or self healing (e.g. in resistant C57BL/6). Several immunomodulatory treatments, like sublethal irradiation or treatment with anti-CD4 [2, 3], have been shown to change the phenotype of BALB/c mice towards resistance; all of these treatments have to be initiated before or within the first few days after infection in order to be effective. The reasons for the different disease outcome in resistant and susceptible mice has been a matter of intensive studies. In the course of these investigations, murine cutaneous *leishmaniasis* became the first model to clearly demonstrate the significance of the Th1/Th2 concept *in vivo*. Herein, we will summarize the key results of these studies.

Because the macrophage is the principle host cell for *L. major*, any attack of the immune system to kill the parasite has to involve this cell. The decisive question therefore is, how to activate the macrophage to use its lethal machinery to kill *Leishmania* . It is now clear that nitric oxide produced by

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the inducible NO synthase (iNOS) is the most important mechanism of the macrophage to kill *Leishmania in vitro* and *in vivo* [4]. Therefore, the actual amounts of iNOS present within macrophages and the capacity to upregulate iNOS expression, determine the outcome of *Leishmania* infection. In accordance with this conclusion, iNOS expression is low in susceptible BALB/c mice and high in resistant C57BL/6 mice during infection with *L. major* [5]. In addition, mice treated with iNOS inhibitors or having a genetic defect in iNOS expression, succumb to infection with *L. major* even, if they are of a resistant genetic background [4, 6]. We have recently shown that IFN a , β is very critical for early production of iNOS [7].

IFNγ is by far the most important cytokine leading to induction of iNOS [4]. In contrast, TGFβ deactivates the macrophage and causes downregulation of iNOS [8]. These findings correlate with the potential of the respective cytokines to influence the parasite-killing potential of macrophages *in vitro* and the course of murine leishmaniasis *in vivo* : macrophages are induced by IFNγ to kill *Leishmania* parasites in an NO-dependent manner [9]. Mice of a resistant background with a genetic defect in IFNγ are susceptible to the infection with *L. major* [10]. In contrast, treatment of *L. amazonensis* - infected susceptible mice with an antibody to TGFβ leads to cure of these animals [11], and there is an inverse correlation between the tissue expression of TGFβ and iNOS [5].

These findings support the conclusion that production of IFNγ after *L. major* infection is critical for cure. Thus, the question arises as to which cells are the early sources of IFNγ . One cell type known to produce this cytokine early in infection, is the NK cell. Therefore, it was important to note that the level of resistance in *L. major* - infected mice correlated with NK cell activity and that depletion of NK cells transiently aggravated the disease [12]. However, T cell deficient nude mice which are of a resistant background and have high NK cell activity, succumb to the infection, but can be saved by transfer of syngeneic T cells [13]. Also, activation of NK cells in susceptible mice does not lead to final cure of the disease. Therefore, NK cells are important effector cells only at the onset of infection. Later,

IFNγ - producing protective T cells take over the burden for successful clearance of the parasites by activated macrophages.

Experimental cutaneous leishmaniosis and Th1/Th2 cells

Soon after the Th1/Th2 concept was first published, it was speculated that the protective T cells might resemble Th1 cells and that the inbred mouse strains might differ in the Th cell subsets expanding during *L. major* infection. First evidence that this assumption might be correct, was provided by cell transfer experiments in which a *L. major* - specific Th1 cell line protected mice from disease, while a Th2 cell line transferred exacerbation [14]. Subsequently, the cytokine mRNAs present in the lesion-draining lymph nodes of *L. major* - infected were determined. In support of the original hypothesis, resistant mice expressed mRNA for Th1 cytokines, while susceptible mice expressed mainly mRNA for Th2 cytokines [15]. Cure of susceptible mice by treatment with anti-CD4 antibodies correlated with upregulation of mRNAs for Th1 cytokines. Further evidence that Th2 cells are responsible for the fatal course of leishmaniasis came from experiments in which Th2 cytokines were manipulated *in vivo*. Neutralisation of the Th2 cell growth factor IL-4 rendered susceptible mice resistant [16], while mast cells, as a source of IL-4, augmented the of *L. major* - induced lesion size [17].

All these results raise the question: why do susceptible mice expand Th2 cells and resistant mice Th1 cells ? In pursuing this question, several investigators tried to adapt the basic knowledge about the generation of Th1 and Th2 cells to the model of murine leishmaniasis. It was shown that a decrease of the parasite - (and therefore antigen-) load shifted BALB/c mice towards the resistant phenotype [18]. B cells as potential APC for Th2 cells exacerbated the disease in susceptible mice [19]. Treatment with IL-12, the main inducer of Th1 cell differentiation, rendered BALB/c mice resistant [20].

While these investigations identified several compontents of the protective immune response *against L. major*, they did not unravel the genes that are responsible for the difference between C57BL/6 and BALB/c mice. Attempts to identify these

genes came from three types of studies: first, it was shown in bone-marrow chimeras that both T cell and non-T cell compartments of susceptible BALB/c mice contribute to their susceptible phenotype [21]. Second, it was recently shown by our group that the IL-4 receptor differs in eight amino acids between BALB/c and C57BL/6 mice resulting in altered IL-4 binding capacities [22]. Importantly, it was shown that, due to this sequence difference, the BALB/c receptor has a significantly elevated dissociation rate for IL-4. This result raises the possibility that IL-4 might have extended bioactivity in BALB/c mice compared to other mouse strains. Third, experiments were performed in the absence of *L. major* parasites using BALB/c and B10.D2 mice carrying an identical TCR transgene reactive with ovalbumin. Because B10.D2 mice are of C57BL/6 origin, but contain MHC genes similar to BALB/c mice, this system allowed the analysis of the T cell response of both types of mice to an identical antigenic peptide. It turned out that transgenic T cells of BALB/c mice primarily produce IL-4, whereas the B10.D2 — derived T cells tend to release IFNγ. The gene responsible for this phenomenon was mapped to chromosome 11 [23]. In earlier studies using backcross breeding and *L. major* infection, a gene (termed Scl-1) responsible for susceptibility was also located to chromosome 11 [24].

A gene located on chromosome 11 that might well be responsible for susceptibility is the gene for the transcription factor interferon-regulatory-factor-1 (IRF-1). We have recently described that C57BL/6 mice with a genetic defect in IRF-1 produce Th2 cytokines, lack IL-12 production and are extremely susceptible to *L. major* infection [25]. Although an attractive candidate, IRF-1 certainly does not account alone for the genetic difference between C57BL/6 and BALB/c mice; it was recently pointed out lthat several different loci, only one of which is located on chromosome 11, are involved in determining resistance to *L. major* [26].

Another difference between BALB/c and C57BL/6 mice that proved to be important for the clinical course of leishmaniasis was a peak of IL-4 synthesis in BALB/c, but not C57BL/6 appearing within 16h after infection with *L. major*. This peak disappeared after 48h and was followed after 72h by another, long-lasting peak of IL-4 production [27]. In C57BL/6 mice, only the later peak was apparent, although at a lower level. Absence of the early peak correlated with resistance in other mouse strains [27]. The peak was produced by NK1.1 negative T cells. It was demonstrated that this T cell population carries a TCR composed of chains of the Vα8/Vβ4 families [28]. Depletion of these T cells converted BALB/c mice into healer mice. The Vα8/Vβ4 positive T cells react with the *L. major* protein LACK which, when expressed in mice as a transgene, leads to tolerance of the mice towards it and, interestingly, to resistance towards *L. major* infection [29]. Although convincing, these results do not exclude that a similar T cell population also exists in resistant C57BL/6 mice. If so, such a population might produce early IFNγ instead of IL-4, due to the genetic difference of C57BL/6 and BALB/c mice. Therefore, at present, it cannot be ruled out that the difference in the early IL-4 production between C57BL/6 and BALB/c is not directly responsible for resistance or susceptibility, but rather is secondary to another as yet unidentified genetic heterogeneity.

REFERENCES
1) Moll H, Fuchs H, Blank C, Rollinghoff M: Langerhans cells transport Leishmania major from the infected skin to the draining lymph node for presentation to antigen- specific T cells. Eur J Immunol ; 23: 1595-1601, 1993.
2) Howard JG, Hale C, Liew FY: Immunological regulation of experimental cutaneous leishmania sis. IV. Prophylactic effect of sublethal irradiation as a result of abrogation of suppressor T cell generation in mice genetically susceptible to Leishmania tropica. J Exp Med ; 153: 557-568, 1981.
3) Titus RG, Ceredig R, Cerottini JC, Louis JA: Therapeutic effect of anti-L3T4 monoclonal antibody GK1.5 on cutaneous leishmania sis in genetically-susceptible BALB/c mice. J Immunol ; 135: 2108-2114, 1985.
4) MacMicking J, Xie QW, Nathan C: Nitric oxide and macrophage function. Annu Rev Immunol ; 15: 323-350, 1997.
5) Stenger S, Thuring H, Rollinghoff M, Bogdan C: Tissue expression of inducible nitric oxide synthase is closely associated with resistance to Leishmania major. J Exp Med ; 180: 783-793, 1994.
6) Wei XQ, Charles IG, Smith A, Ure J, Feng GJ, Huang FP, Xu D, Muller W, Moncada S, Liew FY: Altered immune responses in mice lacking inducible nitric oxide synthase. Nature ; 375: 408-411, 1995.
7) Diefenbach A, Schindler H, Donhauser N, Lorenz E, Laskay T, MacMicking J, Rollinghoff M, Gresser I,

Bogdan C: Type 1 interferon (IFNalpha/beta) and type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. Immunity ; 8: 77-87, 1998.

8) Ding A, Nathan CF, Graycar J, Derynck R, Stuehr DJ, Srimal S: Macrophage deactivating factor and transforming growth factors- beta 1 -beta 2 and -beta 3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-gamma. J Immunol ; 145: 940-944, 1990.

9) Green SJ, Crawford RM, Hockmeyer JT, Meltzer MS, Nacy CA: Leishmania major amastigotes initiate the L-arginine-dependent killing mechanism in IFN-gamma-stimulated macrophages by induction of tumor necrosis factor-alpha. J Immunol ; 145: 4290-4297, 1990.

10) Wang ZE, Reiner SL, Zheng S, Dalton DK, Locksley RM: CD4+ effector cells default to the Th2 pathway in interferon gamma-deficient mice infected with Leishmania major. J Exp Med ; 179: 1367-1371, 1994.

11) Barral Netto M, Barral A, Brownell CE, Skeiky YA, Ellingsworth LR, Twardzik DR, Reed SG: Transforming growth factor-beta in leishmania l infection: a parasite escape mechanism. Science ; 257: 545-548, 1992.

12) Laskay T, Rollinghoff M, Solbach W: Natural killer cells participate in the early defense against Leishmania major infection in mice. Eur J Immunol ; 23: 2237-2241, 1993.

13) Mitchell GF, Curtis JM, Scollay RG, Handman E: Resistance and abrogation of resistance to cutaneous leishmania sis in reconstituted BALB/c nude mice. Aust J Exp Biol Med Sci ; 59: 539-554, 1981.

14) Scott P, Natovitz P, Coffman RL, Pearce E, Sher A: Immunoregulation of cutaneous leishmania sis. T cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. J Exp Med ; 168: 1675-1684, 1988.

15) Heinzel FP, Sadick MD, Mutha SS, Locksley RM: Production of interferon gamma, interleukin 2, interleukin 4, and interleukin 10 by CD4+ lymphocytes in vivo during healing and progressive murine leishmania sis. Proc Natl Acad Sci U S A ; 88: 7011-7015, 1991.

16) Chatelain R, Varkila K, Coffman RL: IL-4 induces a Th2 response in Leishmania major-infected mice. J Immunol ; 148: 1182-1187, 1992.

17) Wershil BK, Theodos CM, Galli SJ, Titus RG: Mast cells augment lesion size and persistence during experimental Leishmania major infection in the mouse. J Immunol ; 152: 4563-4571, 1994.

18) Bretscher PA, Wei G, Menon JN, Bielefeldt Ohmann H: Establishment of stable, cell-mediated immunity that makes " susceptible " mice resistant to Leishmania major. Science ; 257: 539-542, 1992.

19) Sacks DL, Scott PA, Asofsky R, Sher FA: Cutaneous leishmaniasis in anti-IgM-treated mice: enhanced resistance due to functional depletion of a B cell-dependent T cell involved in the suppressor pathway. J Immunol ; 132: 2072-2077, 1984.

20) Heinzel FP, Schoenhaut DS, Rerko RM, Rosser LE, Gately MK: Recombinant interleukin 12 cures mice infected with Leishmania major. J Exp Med ; 177: 1505-1509, 1993.

21) Shankar AH, Titus RG: T cell and non-T cell compartments can independently determine resistance to Leishmania major. J Exp Med ; 181: 845-855, 1995.

22) Schulte T, Kurrle R, Rollinghoff M, Gessner A: Molecular characterization and functional abalysis of murine Interleukin 4 receptor allotypes. J Exp Med ; 186: 1-11, 1997.

23) Gorham JD, Guler ML, Steen RG, Mackey AJ, Daly MJ, Frederick K, Dietrich WF, Murphy KM: Genetic mapping of a murine locus controlling development of T helper 1/T helper 2 type responses. Proc Natl Acad Sci U S A ; 93: 12467-12472, 1996.

24) Mock B, Blackwell J, Hilgers J, Potter M, Nacy C: Genetic control of Leishmania major infection in congenic, recombinant inbred and F2 populations of mice. Eur J Immunogenet ; 20: 335-348, 1993.

25) Lohoff M, Ferrick D, Mittrucker HW, Duncan GS, Bischof S, Rollinghoff M, Mak TW: Interferon regulatory factor-1 is required for a T helper 1 immune response in vivo. Immunity ; 6: 681-689, 1997.

26) Demant P, Lipoldova M, Svobodova M: Resistance to Leishmania major in mice [letter]. Science ; 274: 1392-1393, 1996.

27) Launois P, Ohteki T, Swihart K, MacDonald HR, Louis JA: In susceptible mice, Leishmania major induce very rapid interleukin-4 production by CD4+ T cells which are NK1.1-. Eur J Immunol ; 25: 3298-3307, 1995.

28) Launois P, Maillard I, Pingel S, Swihart KG, Xenarios I, Acha Orbea H, Diggelmann H, Locksley RM, MacDonald HR, Louis JA: IL-4 rapidly produced by V beta 4 V alpha 8 CD4+ T cells instructs Th2 development and susceptibility to Leishmania major in BALB/c mice. Immunity ; 6: 541-549, 1997.

29) Julia V, Rassoulzadegan M, Glaichenhaus N: Resistance to Leishmania major induced by tolerance to a single antigen. Science ; 274: 421-423, 1996.