Introduction à la biologie des parasites
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Invited review

The murine model of infection with *Leishmania major* and its importance for the deciphering of mechanisms underlying differences in Th cell differentiation in mice from different genetic backgrounds

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Abstract

Mice from the majority of inbred strains are resistant to infection by *Leishmania major*, an obligate intracellular protozoan parasite of macrophages in the mammalian host. In contrast, mice from BALB strains are unable to control infection and develop progressive disease. In this model of infection, genetically determined resistance and susceptibility have been clearly shown to result from the appearance of parasite-specific $CD4^+$ T helper 1 or T helper 2 cells, respectively. This murine model of infection is considered as one of the best experimental systems for the study of the mechanisms operating in vivo at the initiation of polarised T helper 1 and T helper 2 cell maturation. Among the several factors influencing Th cell development, cytokines themselves critically regulate this process. The results accumulated during the last years have clarified some aspects of the role played by cytokines in Th cell differentiation. They are providing critical information that may ultimately lead to the rational devise of means by which to tailor immune responses to the effector functions that are most efficient in preventing and/or controlling infections with pathogens.

Keywords: Th1/Th2 responses; Infection with *Leishmania*; Cytokines

1. The murine model of infection with *Leishmania major*

Upon experimental infection with *L. major*, distinct features of the spectrum of clinical manifestations seen in patients with cutaneous leishmaniasis can be reproduced in inbred mice of different genetic backgrounds (Behin et al., 1979; Mitchell et al., 1981). Mice from the majority of inbred strains (C3H/He, CBA, C57BL/6, 129Sv/Ev) develop locally cutaneous lesions, which spontaneously resolve. These mice do not develop lesions after a second inoculation of *L. major* and belong to the resistant phenotype. Mice from a few strains (BALB) develop severe and uncontrolled lesions without becoming immune to re-infection and are representative of the susceptible phenotype (Mitchell et al., 1981). This murine model of infection has been used to characterise the immune responses developing in both resistant and susceptible mice.

2. Expression of the resistant or susceptible phenotype following infection with *L. major* results from the maturation of $CD4^+$ T helper 1 or T helper 2 response, respectively

Two functionally distinct $CD4^+$ T cell subsets, T helper 1 (Th1) and T helper 2 (Th2), distinguishable by the pattern of cytokines they produce upon stimulation, have been described in the late 1980s (Mosmann and Coffman, 1989). Th1 cells are characterised by secretion of IFN-γ and lymphotoxin that are known to activate host defences against intracellular pathogens, while Th2 cells produce IL-4, IL-5, and IL-13 (Cherwinski et al., 1987) that favour the development of humoral responses protecting against extracellular pathogens (Abbas et al., 1996).

The murine model of infection with *L. major* provided the first correlation in vivo between (1) the development of protective immunity and an expansion of Th1 $CD4^+$ T cells in resistant mice and (2) the expression of progressive disease and the development of a $CD4^+$ Th2 cell response in susceptible mice (Locksley et al., 1987; Scott et al., 1988; Heinzel et al., 1989).

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3. Cytokines controlling parasite growth

Activation of macrophages to a parasiticidal state by IFN-γ is the main anti-*Leishmania* effector mechanism. Indeed, IFN-γ has been demonstrated to increase the synthesis of inducible nitric oxide synthase (iNOs) leading to the L-arginine dependent production of reactive nitrogen radicals toxic for the parasite (Green et al., 1990; Liew et al., 1990; Mauël et al., 1991; Assreuy et al., 1994). The role of IFN-γ in conferring resistance to infection with *L. major* was demonstrated by showing that genetically resistant mice lacking either the IFN-γ or the IFN-γ receptor gene are unable to control parasite growth (Wang et al., 1994; Swihart et al., 1995). The IFN-γ mediated activation of macrophages can be regulated by other cytokines produced by Th2 cells such as IL-4 and IL-10 (Liew et al., 1989). Furthermore, TGF-β, a well-known deactivating cytokine, interferes with NO production in BALB/c mice (Stenger et al., 1994).

Although IFN-γ mediated activation of macrophages to a microbicidal stage is the main mechanism of destruction of *L. major*, the CD4 Th1 cell mediated cytotoxicity depending on a functional Fas–FasL pathway (Ju et al., 1994; Ramsdell et al., 1994; Stalder et al., 1994) could also contribute to the healing of lesions in resistant mice infected with *L. major*. Indeed, in contrast to wild-type C57BL/6 mice, C57BL/6 mice lacking either a functional FasL or Fas were unable to heal lesions induced by *L. major* in spite of the fact that they developed a $CD4^+$ Th1 response and their macrophages produced normal levels of reactive nitrogen in response to IFN-γ in vitro. Restoration of a functional Fas–FasL pathway of cytotoxicity by exogenous FasL in FasL-deficient mice, allowed complete resolution of the cutaneous lesions, clearly demonstrating that a functional Fas–FasL pathway is one of the components necessary for healing (Conceiçao-Silva et al., 1998; Huang et al., 1998). The apoptotic death of infected macrophages could be hypothesised to result in a decrease in the ratio of infected macrophages to IFN-γ producing Th1 cells, thus increasing the efficiency of macrophage activation to a microbicidal state.

4. Factors influencing the development of Th1 or Th2 $CD4^+$ T cells

Th1 and Th2 $CD4^+$ T cells develop from common naïve $CD4^+$ T cell precursors (Röcken et al., 1992; Kamogawa et al., 1993). Several parameters have been reported to influence the pathway of differentiation of $CD4^+$ T cell precursors, including the type of antigen presenting cells (APCs; Moser and Murphy, 2000), the nature of the co-stimulatory signals (Kuchroo et al., 1995), the extent of T cell receptor (TCR) engagement (Pfeiffer et al., 1995), the dose of antigen (Hosken et al., 1995), the route of antigen administration (Guéry et al., 1996), the number of cell division (Bird et al., 1998). Among these different T cell polarising signals, cytokines have been recognised as crucial inducers of $CD4^+$ Th1 and Th2 cell differentiation. In a recent review, the contribution of these various parameters to the development of polarised Th1 and Th2 responses in vivo following infection with *L. major* has been remarkably discussed (Sacks and Noben-Trauth, 2002).

4.1. The role of cytokines in the development of polarised $CD4^+$ Th2 responses

4.1.1. Role of IL-4

Studies in vivo using the murine model of infection with *L. major* have established that IL-4 during the early stages of infection has an important role on the subsequent development of specific $CD4^+$ Th2 cells. The first evidence was that administration of anti-IL-4 antibodies to BALB/c mice at the onset of infection abrogated Th2 polarisation, allowed an expansion of Th1 cells and consequently led to resistance to infection (Sadick et al., 1990). The importance of IL-4 has been further established by results showing that many other immune interventions, effective only at the initiation of infection, that are able to favour the development of a Th1 response to *L. major* in BALB/c mice diminish IL-4 production during the first week of infection (Müller et al., 1992; Heinzel et al., 1993b; Sypek et al., 1993; Corry et al., 1994). Direct evidence has been obtained that BALB/c mice, in contrast to resistant mice, produce a burst of IL-4 extremely rapidly in response to infection with *L. major* (Launois et al., 1995). Indeed, 16 h after subcutaneous infection, BALB/c mice exhibited a peak in IL-4 mRNA expression in the draining lymph nodes, before returning to baseline levels by 48 h. Importantly, this IL-4 production occurred during the period when neutralising IL-4 antibodies are capable of redirecting protective Th1 development in BALB/c mice (Sadick et al., 1990). From day 5, a second wave of IL-4 mRNA was observed that remained elevated during the entire course of infection, reflecting the Th2 cell differentiation normally observed in these susceptible mice (Launois et al., 1995). In contrast, no increase in IL-4 mRNA expression was observed in C57BL/6 resistant mice during the first 2 days of infection with *L. major* (Launois et al., 1995).

Results obtained by our laboratory have documented that there was a short period of time of less than 48 h after infection during which the IL-4, produced as a result of the 16 h burst in IL-4 transcription, must be biologically active in order to enforce subsequent Th2 cell development. During that time, IL-4 rendered *L. major*-specific $CD4^+$ T cells unresponsive to IL-12 (Launois et al., 1997b). The IL-12 responsiveness of lymphocytes requires a high affinity

IL-12R. Two IL-12R subunits, IL-12Rβ1 and IL-12Rβ2 chains, have been described (Presky et al., 1996) and the IL-12 Rβ2 is essential for IL-12 signalling (Szabo et al., 1995). Interestingly, extinction of IL-12 signalling in BALB/c mice is due to a rapid down-regulation of IL-12Rβ2-chain mRNA expression in $CD4^+$ T cells that occurred 48 h after infection and was observed at least up to day 8. Neutralisation of the IL-4 produced in BALB/c mice during the first days of infection resulted in maintenance of the IL-12Rβ2 chain mRNA expression. Resistant C57BL/6 mice, which do not mount an early IL-4 mRNA burst following infection with *L. major* (Launois et al., 1995), maintained the expression of the IL-12Rβ2 chain on their specific $CD4^+$ T cells, that were responsive to IL-12 (Himmelrich et al., 1998). Thus, IL-4 rapidly produced after infection with *L. major* inhibits IL-12Rβ2 chain expression on $CD4^+$ T cells resulting in a state of unresponsiveness to IL-12 and a Th2 cell development. However, since it has been recently documented that transgenic BALB/c mice expressing a transgenic IL-12Rβ2 chain display progressive lesions and mount a Th2 cell response after infection with *L. major* similar to wild-type BALB/c mice, the exact role of the down-regulation of IL-12Rβ2 chain expression in Th2 differentiation remains to be determined (Nishikomori et al., 2001; Fig. 1).

Quantification of IL-4 mRNA in $CD4^+$ T cell populations purified from lymph nodes of BALB/c mice 16 h after infection directly identified $CD4^+$ T cells as the source of IL-4 (Launois et al., 1995). Analysis of the TCR Vβ and Vα usage of $CD4^+$ T cells producing IL-4 within the first day of infection with *L. major* demonstrated that all of the IL-4 mRNA present at this time was produced by $CD4^+$ T cells that expressed the Vβ4 and the Vα8 TCR chains (Launois et al., 1997a). The contribution of these Vβ4–Vα8 $CD4^+$ T cells to the early burst of IL-4 mRNA expression in BALB/c mice in response to infection was confirmed by experiments showing that this response was absent in BALB/c mice rendered selectively deficient in the $V\beta4^+CD4^+$ T cell population by mouse mammary tumour viruses (MMTV) infection (Launois et al., 1997a). The MMTV(SIM type) and MMTV(SW type) encode a superantigen that ultimately leads to systemic deletion of $CD4^+$ T cells expressing the Vβ4 or Vβ6 TCR chain, respectively (Held et al., 1992; Maillard et al., 1996). Thus, in contrast to wild-type BALB/c mice or BALB/c mice deficient in $V\beta6^+CD4^+$ T cells as a result of neonatal exposure to MMTV(SW), BALB/c mice deficient in $V\beta4^+CD4^+$ T cells by prior infection with MMTV(SIM) were not able to generate early IL-4 transcripts in $CD4^+$ T cells following infection with *L. major*, developed a Th1 response and were resistant to infection (Launois et al., 1997a). Noteworthy, administration of exogenous IL-4 to Vβ4-deficient BALB/c mice only during the first 64 h of infection restored Th2 cell development and susceptibility to infection (Himmelrich et al., 2000).

Cloned $CD4^+$ T cell lines and hybridoma recognising the *Leishmania* homologue of mammalian RACK1 designated LACK (*Leishmania* Activated c Kinase) preferentially expressed the Vβ4–Vα8 TCR chains (Mougneau et al., 1995). Interestingly, the LACK antigen from *L. major* also

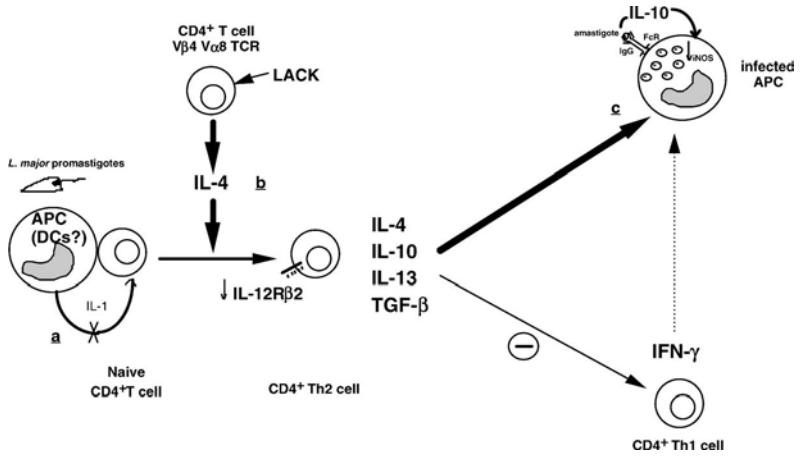

Fig. 1. Some of the events that could contribute to T helper 2 cell development in susceptible mice infected with *Leishmania major*. (a) Impaired IL-1 production by dendritic cells infected with *L. major* favours T helper 2 maturation and inhibits T helper 1 cell development. (b) Early IL-4 production by LACK-reactive $CD4^+$ T cells instructs T helper 2 cell maturation and prevents IL-12 signalling. (c) T helper 2 cytokines inhibit macrophage activation by IFN-γ (see text for references).

induced early IL-4 mRNA expression in the same Vβ4–Vα8 population of BALB/c mice (Launois et al., 1997a). This response was driven by a single dominant $I\text{-}A^d$-restricted T cell epitope in the fourth WD domain of LACK, comprising amino acid residues 156–173 since injection of truncated LACK recombinant protein missing the 41 aa that included the 18 aa epitope (aa 156–173) did not induce IL-4 mRNA expression (Launois et al., 1997a). The critical role of this immunodominant epitope from LACK and the IL-4 that it triggers, in subsequent Th2 cell development, was confirmed using LACK altered peptide ligands that differ by a single amino acid from natural $I\text{-}A^d$ restricted epitope. Indeed, the induction of a specific unresponsive state in LACK-reactive Vβ4–Vα8 $CD4^+$ T cells following treatment of BALB/c mice with altered LACK proteins antagonises early IL-4 response to the wild-type LACK epitope, inhibits Th2 cell development, redirects Th1 cell maturation and results in long-term protection (Pingel et al., 1999). These data are also supported by results which have shown that BALB/c mice tolerant to LACK, as a result of the transgenic expression of this molecule in the thymus under major histo-compatibility complex class II promoters, are resistant to *L. major* and develop a Th1 response (Julia et al., 1996).

In contrast to susceptible BALB/c mice, C57BL/6 and other resistant mice did not mount an early IL-4 response following infection with *L. major* or injection of LACK (Launois et al., 1995). However, recent findings have demonstrated that neutralisation of either IL-12 or IFN-γ in C57BL/6 mice at the initiation of infection allows the expression of a rapid IL-4 response to *L. major* or LACK (Launois et al., 2002). Strikingly, this early IL-4 response in C57BL/6 mice also occurred in $CD4^+$ T cells that express the Vβ4–Vα8 TCR chains that were also reactive to LACK. Analysis of the epitopes recognised by these Vβ4–Vα8 $CD4^+$ T cells from C57BL/6 mice is being carried out. Preliminary results clearly show that C57BL/6 Vβ4–Vα8 $CD4^+$ T cells recognise an $I\text{-}A^b$-restricted epitope different from the $I\text{-}A^d$-restricted $LACK_{(156-173)}$ peptide seen by BALB/c Vβ4–Vα8 $CD4^+$ T cells. Noteworthy, the IL-4 produced during the early stage of infection in these mice accounted at least in part for the increased susceptibility of resistant mice treated with either anti-IFN-γ or anti-IL-12 (Scott, 1991; Heinzel et al., 1995; Launois et al., 2002).

Collectively, these results imply that the role of these Vβ4–Vα8 $CD4^+$ T cells is to provide the IL-4 necessary for Th2 maturation and suggest that in BALB/c mice, a single antigen (LACK) from this highly complex micro-organism drives the early IL-4 response that underlies subsequent Th2 cell maturation resulting in progressive disease. However, recent results using MHC/peptides and IL-4 reporter mice have shown comparable precursor frequency and expansion of IL-4 expression between B10.D2 (resistant phenotype) and BALB/c. Although this study confirmed the pathogenic role of LACK reactive cells and the IL-4 they produced in susceptible mice, it suggests that IL-4 might not be the only signal necessary for Th2 differentiation in susceptible mice (Stetson et al., 2002). This conclusion is also supported by the conflicting results obtained using IL-4-deficient susceptible mice. One group reported that these mice still express a susceptible phenotype to infection with *L. major* (Noben-Trauth et al., 1996) and that IL-4-deficient mice lose their IL-12 responsiveness equally as do wild-type BALB/c mice (Kropf et al., 1997). Furthermore, susceptibility to infection with *L. major* could be prevented by administration of IL-12 to these IL-4-deficient BALB/c mice (Kropf et al., 1997). In contrast, another group reported that these IL-4-deficient BALB/c mice expressed a resistant phenotype to infection with *L. major* (Kopf et al., 1996). Understanding the basis of such conflicting data might rely on the assessment of the role of other Th2 cytokines such as IL-13, IL-10 and TGF-β in the development of Th cell responses during infection with *L. major*.

4.1.2. Role of IL-13

Several observations indicate that IL-13, which shares biological functions with IL-4 (Chomarat and Banchereau, 1998), is involved in susceptibility to infection with *L. major*. Infected $IL\text{-}4^{-/-}$ BALB/c mice treated with IL-13Rα2 fusion protein, that blocks the biological activity of IL-13, are totally resistant to infection with *L. major* (Kropf et al., 1999). Furthermore, the use of IL-13 deficient mice and IL-13 transgenic mice demonstrated that IL-13 is important for the generation of Th2 cells. Indeed, $IL\text{-}4^{-/-}$ C57BL/6 mice with the transgenic expression of IL-13 failed to control infection with *L. major* (Matthews et al., 2000). Interestingly, some additive effects of deletion of IL-4 and IL-13 were reported. Furthermore, if $IL\text{-}4^{-/-}$ BALB/c mice infected with *L. major* IR173 controlled only partially infection, $IL\text{-}4R\alpha^{-/-}$ mice were fully resistant to infection (Noben-Trauth et al., 1999). Thus, since IL-4Rα is a component of the IL-13R (Hilton et al., 1996), these results support the importance of IL-13 in the expression of a susceptible phenotype to *L. major*. The fact that IL-13 is a factor of susceptibility to infection with *L. major* is not surprising. Indeed, genetic mapping of loci involved in the outcome of infection with *L. major* demonstrated that one of the loci involved maps to chromosome 11 which contains the IL-4/IL-13 gene cluster (Beebe et al., 1997; Mock et al., 1993; Roberts et al., 1993).

4.1.3. Role of IL-10

The fact that $IL\text{-}4R\alpha^{-/-}$ mice developed progressive disease following infection with *L. major*, strain LV39 suggests that although IL-4 and IL-13 biological activity is lacking, these mice could be, under some circumstances, fully susceptible (Noben-Trauth et al., 1999). Consequently, it is possible that mechanisms or cytokines other than IL-4 and IL-13 can promote susceptibility to infection. The fact that the lesions size and number of parasites are reduced in $IL\text{-}4R\alpha^{-/-}$ mice treated with anti-IL-10 mAbs or in double $IL\text{-}4R\alpha^{-/-}$ $IL\text{-}10^{-/-}$ mice infected with LV39 indicate that

IL-10 could play a role in susceptibility to infection with *L. major* (Noben-Trauth et al., 2003). Furthermore, it has been established that LV39 requires a higher concentration of IFN-γ than IR173 to be killed. Thus, IL-10 might be as important as IL-4 and IL-13 in the development of susceptibility particularly after infection with strains relatively resistant to IFN-γ such as LV39 (Noben-Trauth et al., 2003).

Although treatment with anti-IL-10 mAbs at the onset of infection had little effect either on Th maturation or development of lesions (Chatelain et al., 1999), IL-$10^{-/-}$ BALB/c mice were more resistant to *L. major* than wild-type mice (Kane and Mosser, 2001). The IL-10 production by macrophages following interaction of IgG bound to amastigotes with the FcR has been suggested as a molecular mechanism behind the role of IL-10 (Kane and Mosser, 2001). In this context, IL-10 transgenic mice on resistant background are more susceptible to infection with *L. major* by still mounting a Th1 response (Groux et al., 1999). Altogether these results suggest that IL-10 could also play a role in susceptible mice infected with *L. major*. Interestingly, it has been recently shown that IL-10 is also implicated in the persistence of *Leishmania* in healed lesions of resistant C57BL/6 mice since complete elimination of parasites occurred in these mice only when the IL-10 signaling pathway was abrogated with anti-IL-10R mAbs (Belkaid et al., 2001).

4.1.4. Role of TGF-β

TGF-β is a pleiotropic cytokine known to inhibit the differentiation of naïve T cells into either Th1 or Th2 cells (Letterio and Roberts, 1998). The transgenic expression of the dominant negative TGF-receptor II, exclusively on T cells of susceptible BALB/c mice, thereby blocking TGF-signalling in T cells, results in resistance to infection with *L. major* (Gorelik et al., 2002). However, although these mice developed increased production of Th1 cytokine at a higher level than resistant C57BL/6 mice, Th2 cytokine production was also enhanced. These results confirm earlier observations which demonstrated that TFG-β plays a role in the progression of infection with *Leishmania braziliensis* or *Leishmania amazonensis*. Indeed, administration of TGF-β to C57BL/6 infected with *L. braziliensis* or *L. amazonensis* resulted in progressive disease whereas neutralisation of TGF-β in BALB/c mice inhibited the progression of lesions (Barral-Netto et al., 1992). The effect of TGF-β has been associated with an inhibition of the anti-parasiticidal functions of macrophages. In this context, TFG-β suppresses production of nitric oxide by macrophages leading to enhanced progression of lesions (Li et al., 1999). However, TGF-β could also act directly on T cells since it inhibits the expression of T-bet, a transcription factor central to Th1 differentiation (Gorelik et al., 2002). Thus, TGF could act, not only at the effector phase of the Th response, but also on the differentiation of Th cell precursors cells.

4.1.5. Role of IL-2

IL-2 is a 15.5 kDa glycoprotein mainly produced by activated T cells (i.e. Th1 cells, Mosmann and Coffman, 1989), although activated B cells produce small amounts of IL-2 (Harris et al., 2000). IL-2 acts on a large variety of target cells including T and B lymphocytes, NK cells, and macrophages/monocytes (Smith, 1988). This cytokine plays a central role in the regulation of immune responses. The IL-2-dependence of IL-4 production by $CD4^+$ T cells is controversial. Indeed priming of $CD4^+$ T cells for Th2 differentiation although dependent upon IL-4 has been shown to also require IL-2 (Swain et al., 1990; Seder et al., 1994). However, Th2 cell development appears possible in the absence of IL-2 (Schorle et al., 1991; Sadlack et al., 1994). In this context, we initiated studies to directly assess the role of IL-2 on Th2 cell maturation of BALB/c mice infected with *L. major*. Preliminary results have documented an increase in IL-2 transcription in draining lymph nodes from susceptible BALB/c that even preceded the early IL-4 response to *L. major* (Gumy et al., unpublished results). The IL-2 produced as a result of this early IL-2 mRNA burst was found necessary for the expression of the rapid burst of IL-4 transcripts since neutralisation of IL-2 during the first days of infection redirected Th1 cell maturation and resistance to *L. major* in these otherwise susceptible mice. These effects of neutralising IL-2 antibody were related to its ability to interfere with the generation of a rapid IL-4 transcriptional burst. Altogether these results suggest that IL-2 is necessary for Th2 cell development resulting in susceptibility to infection with *L. major* in BALB/c mice. In this context, it has been previously shown that weekly treatment of BALB/c mice with anti-IL-2 mAbs resulted in resistance to infection and reduced IL-4 production by specifically stimulated lymph node cells in vitro (Heinzel et al., 1993a). However, studies comparing IL-2 production between susceptible and resistant mice are presently being undertaken.

4.2. The role of cytokines in the development of polarised $CD4^+$ Th1 responses

4.2.1. Role of IL-12

In vitro, different cytokines, such as IL-12, IFN-γ itself, IL-18 and more recently IL-23 and IL-27, have been shown to play a role in the optimal production of IFN-γ by Th1 cells. IL-12 is a cytokine produced by several cell types such as dendritic cells (DCs), macrophages, polymorphonuclear cells and B cells (Trinchieri, 1995). IL-12 is a 70 kDa heterodimer (p70) consisting of two subunits, p40 and p35 which are both required for biological activity (Wolf et al., 1991). The role of IL-12 in vivo has been extensively studied in the murine model of infection with *L. major*. Exogenous IL-12 injected during the first week of infection with *L. major* in susceptible BALB/c mice resulted in the development of a $CD4^+$ Th1 response associated with decreased production of IL-4 by lymph node T cells in vitro

and allowed these otherwise susceptible BALB/c mice to resolve their lesions (Heinzel et al., 1993b; Sypek et al., 1993). Conversely, neutralisation of IL-12 by polyclonal anti-sera against IL-12 in resistant mice led to an increase in the production of IL-4 and to the generation of a susceptible phenotype (Heinzel et al., 1993b; Sypek et al., 1993). The importance of IL-12 in Th1 cell development during infection with *L. major* has been further tested using mice genetically deficient in either the p35 or p40 subunits of IL-12. These mice, generated on a resistant genetic background, developed progressive lesions. This was associated with an important increase in the levels of IL-4 mRNA in their lymph nodes reaching values comparable to those observed in similarly infected susceptible BALB/c mice (Mattner et al., 1996; Fig. 2).

The role of IL-12 in Th1 cell development has been further documented by the fact that genetic deficiency of a transcription factor (T-bet, Szabo et al., 2002) or co-stimulatory molecules (CD40–CD40L; Campbell et al., 1996; Kamanaka et al., 1996), which are involved in a Th1 response, rendered resistant mice susceptible to infection with *L. major*. Indeed, these deficient mice exhibited a down-regulation of the IL-12 production associated with maturation of a typical Th2 cell response. However, the precise timing of IL-12 production in vivo following infection with *L. major* is still unclear. Results from one study have shown that IL-12 transcripts were neither detected in susceptible nor in resistant mice during the first 10 days after infection (Reiner et al., 1994). In another study, some IL-12 production could be detected in lymph nodes from either susceptible (BALB/c) or resistant mice (C3H), but not in mice from another resistant strain (C57BL/6, Vieira et al., 1994; Scharton-Kersten et al., 1995). Since functional expression of IL-12R has been shown to be enhanced in the presence of IL-12, suggesting that IL-12 regulates its own receptor (Sinigaglia et al., 1999), it has been proposed that the IL-12 produced during infection with *L. major* is able to induce its own receptor at the surface of naïve cells allowing their further differentiation towards the Th1 phenotype (Sinigaglia et al., 1999).

4.2.2. Role of IFN-γ

There are also some debates concerning the importance of IFN-γ in favouring Th1 cell response during infection with *L. major*. Administration of anti-IFN-γ antibodies to resistant mice (C57BL/6 or C3H/He) within the first 2 days of infection rendered these mice unable to resolve their lesions and led to the appearance of a Th2 response (Belosevic et al., 1989; Scott, 1991; Launois et al., 2002). In agreement with these findings, IFN-γ-deficient C57BL/6 mice developed a Th2 response after *L. major* infection (Wang et al., 1994). In contrast, resistant 129/Sv/Ev mice deficient for the binding chain of the IFN-γ receptor (IFN-γR), which are also exquisitely susceptible to infection, still developed a Th1 response (Swihart et al., 1995). IFN-γ could influence Th1 differentiation by enhancing either IL-12 expression or the responsiveness of naïve T cells to IL-12. Since it has been shown that IFN-γ could activate the IL-12p40 gene promoter in monocytic cells at least in human (Ma et al., 1996), IFN-γ might effectively up-regulate IL-12 production. Furthermore, since IFN-γ activation of T-bet through signal transducer and activator of transcription (STAT)-1 signaling induces IL-12 Rβ2 chain expression on $CD4^+$ T cells, IFN-γ could influence the IL-12 responsiveness of these cells (Afkarian et al., 2002).

Recently, a new model for Th1 differentiation was suggested based on the respective role of IL-12 and IFN-γ

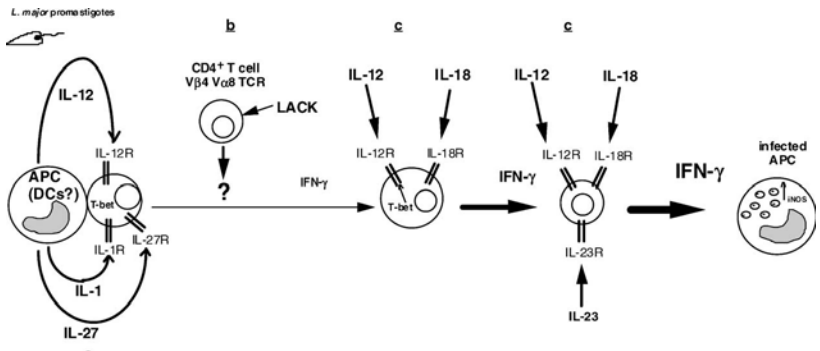

Fig. 2. Some of the events that could contribute to T helper 1 cell development in resistant mice infected with *Leishmania major*. (a) Early production of IL-1 by dendritic cells and IL-27 favours T helper 1 cell maturation. (b) Down-regulation of IL-4 production and possible release of other cytokines(?) by LACK-reactive $CD4^+$ T cells prevents T helper 2 cell development. (c) Maintenance of IL-12R expression (T-bet; IL-18; IL-23) strengthen T helper 1 maturation and commitment (see text for references).

on the activation of STAT-4 and T-bet. Indeed, optimal T-bet expression is first induced by both TCR triggering and IFN-γ activation through the STAT-1 signaling pathway (Afkarian et al., 2002). Since T-bet induces IL-12Rβ2 chain on $CD4^+$ T cells (Afkarian et al., 2002), it regulates their IL-12 responsiveness through STAT-4 activation (O'Shea and Paul, 2002).

However and interestingly, there is no evidence for major differences in IFN-γ production between resistant and susceptible mice during the early phase of infection. Thus, other mechanisms than simply differences in levels of IFN-γ production are needed to explain the role of IFN-γ in the Th1 cell differentiation during infection with *L. major*.

4.2.3. Role of other cytokines (IL-18, IL-23, IL-27 and IL-1)

In addition to IL-12, other cytokines such as IL-18, IL-23 and IL-27 favour IFN-γ production by $CD4^+$ T cells. IL-18 is a member of the IL-1 cytokine family and is produced by monocytic cells. Although IL-18 acts synergistically with IL-12 on production of IFN-γ by Th1 cells (Robinson et al., 1997), it is not sufficient by itself to instruct protective immunity to *L. major*. Indeed, IL-$18^{-/-}$ resistant C57BL/6 mice spontaneously heal their lesions and mount a typical Th1 response after infection with *L. major* (Monteforte et al., 2000). Interestingly, the fact that IL-12 up-regulates IL-18R suggests that IL-18 is a cofactor of Th1 cell development (Ahn et al., 1997; Tomura et al., 1998).

IL-23, which is preferentially produced by activated DCs, is constituted of two subunits, a novel protein p19 related to IL-12p35, and the p40 subunit of IL-12 (Oppmann et al., 2000). Even though IL-23 has been reported to selectively induce proliferation of memory T cells, it does not appear to directly act on naïve $CD4^+$ T cells suggesting that this cytokine is not involved in the differentiation of naïve $CD4^+$ T cells (Oppmann et al., 2000). The fact that IL-$12p35^{-/-}$ mice that are unable to produce IL-12 are totally susceptible to infection with *L. major* is in accordance with this hypothesis since these mice should still be able to produce IL-23 (Mattner et al., 1996). However, IL-23 could act as an IL-12 cofactor on differentiated Th1 effector to maintain an optimal IFN-γ production (Robinson and O'Garra, 2002).

IL-27, produced by APCs, is a heterodimer constituted of an IL-12p40 related protein (EBI3) and p28, an IL-12p35 related protein (Pflanz et al., 2002). The fact that IL-27 induces proliferation in synergy with IL-12 in naïve but not in memory $CD4^+$ T cells (Pflanz et al., 2002) suggests that IL-27 is an important factor for Th1 differentiation. In this context, it has been shown that WSX-$1^{-/-}$ mice, deficient for the IL-27R, on the resistant C57BL/6 background mount a Th2 cell response and are more susceptible to infection with *L. major* than wild-type mice although the lesions never reached the magnitude of those obtained in BALB/c mice (Yoshida et al., 2001). Furthermore, these WSX-$1^{-/-}$ mice showed impaired production of IFN-γ only during the early phases of infection suggesting that IL-27 acts at the initiation stage of Th1 development (Yoshida et al., 2001). Finally, the effect of IL-27 on Th1 differentiation does not appear dependent of IL-12 since the IL-12R is induced in splenocytes in the absence of IL-27 (Chen et al., 2000).

Recently, it has been demonstrated that $CD11b^+$ DCs from B10.D2 expressed more IL-1β than those from BALB/c mice suggesting that IL-1β is one parameter involved in the ability of DCs from B10.D2 mice to induce Th1 proliferation (Filippi et al., 2003). In this context, it has been also demonstrated that IL-1α production was more important in C57BL/6 mice than in BALB/c mice (von Stebut et al., 2003). The facts that mice deficient for the IL-1 type I receptor developed Th2 responses after *L. major* infection (Satoskar et al., 1998) and that BALB/c mice treated with IL-1α or IL-1β developed more Th1 response further support a role of IL-1 in Th1 cell maturation (Filippi et al., 2003; von Stebut et al., 2003). Thus the cellular and molecular basis for the effect of IL-1 on the development of Th1 responses has to be investigated.

4.2.4. Role of IL-4

Although the effect of IL-4 on Th2 differentiation is well admitted, this concept has been recently challenged by results obtained with IL-4-transgenic or IL-4-deficient mice and neutralising IL-4 antibodies. These experiments suggest that, paradoxically, IL-4 may promote Th1 development and the initiation of delayed type hypersensibility. For example, when infected with certain strains of *L. major* or *Candida albicans*, IL-4-deficient mice show defects in developing Th1 responses (Noben-Trauth et al., 1996; Mencacci et al., 1998). Similarly, a critical role for IL-4 and STAT6 (a molecule indispensable for IL-4 signalling) was shown in the development of efficient Th1 responses to haptens (Salerno et al., 1995; Traidl et al., 1999; Yokozeki et al., 2000), auto-antigens, allo-antigens and even tumour antigens (Tepper et al., 1989; Golumbek et al., 1991; Schuler et al., 1999; Bagley et al., 2000; Radu et al., 2000). Promotion of Th1 cell development by IL-4 has been proposed to stem from the capacity of this cytokine to induce IL-12 production by DCs (Hochrein et al., 2000; Kalinski et al., 2000; Biedermann et al., 2001; Ebner et al., 2001). These data suggest a model in which, when available only during the initial period of DCs activation preceding T cell stimulation, IL-4 instructs DCs to produce IL-12 and consequently induces Th1 cell development. In contrast, extension of the IL-4 availability to the period of T cell activation through their specific TCR results in Th2 cell maturation. Thus, we have recently demonstrated that treatment with IL-4 during a period preceding T cell activation (8 h) resulted in a rapid increase of IL-12 mRNA expression in DCs. Furthermore, this treatment rendered BALB/c mice fully resistant to infection with *L. major* and redirected Th1 cell development. Strikingly, the early IL-4 mRNA expression normally seen in LACK-reactive Vβ4–Vα8 $CD4^+$ T cells was abrogated in BALB/c mice receiving

IL-4 during the period preceding T cell activation. This IL-4 suppression was the direct consequence of the IL-12 produced in DCs (Biedermann et al., 2001). In contrast, extension of IL-4 treatment to the period of parasite T-cell priming reversed the resistance and redirected Th2 cell development (Biedermann et al., 2001). In conclusion, when present only during the initial activation of APC by *L. major* in vivo, IL-4 instructs the differentiation of $CD4^+$ T cells toward a Th1 phenotype and establishes resistance to *L. major* in susceptible BALB/c mice. The opposite effects that IL-4 could exert on Th differentiation thus vary according to the cells targeted for IL-4 signalling. Therefore, IL-4 should also be considered as a potential cytokine involved in Th1 cell development.

4.2.5. LACK-reactive Vβ4–Vα8 $CD4^+$ T cells are sensitive to regulatory processes

In the past few years, the concept that subpopulations of T cells are specialised in the suppression of immune responses has been revisited. Considerable attention has been given to a minor subpopulation of $CD4^+$ T cells constitutively expressing CD25, the α-chain of the IL-2 receptor. Both in mice and humans, these cells, named regulatory T cells (Treg cells) have been shown capable of suppressing the proliferation of other T cell populations (Shevach, 2002).

The possibility of modulating the rapid IL-4 response by treatment with either exogenous IL-12, IFN-γ (Launois et al., 1995) or anti-IL-2 mAbs (Gumy et al., unpublished results) suggested that LACK-specific $CD4^+$ T cells are not irreversibly committed to IL-4 production. This contention is supported by the demonstration of the functional plasticity of these cells in terms of cytokines production (Maillard et al., 2001). Together these results suggested that these Vβ4–Vα8 $CD4^+$ T cells were sensitive to regulatory processes. Therefore, studies have been undertaken to determine whether or not $CD4^+CD25^+$ regulatory T cells regulate the early IL-4 production. The results obtained provided evidence that $CD4^+CD25^+$ T cells negatively regulate the magnitude of the early IL-4 response to *L. major* in BALB/c mice as well as the importance of subsequent Th2 cell maturation (Aseffa et al., 2002). Furthermore, $CD4^+CD25^+$ T cells suppressed the development of disease after infection with *L. major* in SCID mice reconstituted with naïve $CD4^+CD25^-$ T cells (Aseffa et al., 2002; Xu et al., 2003). In conclusion, the data indicate that regulatory T cells, previously shown as being important during auto-immune responses (Shevach, 2002), may also regulate harmful immune responses to infectious pathogens.

Remarkably, studies from others strongly suggested that in resistant C57BL/6 mice, $CD25^+$ regulatory T cells were essential for parasite persistence in immune mice and maintenance of memory responses to *L. major* (Belkaid et al., 2002). In this system, it appears that suppression operates via IL-10-dependent and independent mechanisms.

5. Conclusions

The murine model of infection with *L. major* has demonstrated the crucial role of functional $CD4^+$ Th1 and Th2 cell populations in the outcome of infection and has provided important information concerning the conditions favouring polarisation of Th responses in vivo. Although Th cell differentiation in human infection with *Leishmania* is not as clear as in mice infected with *L. major*, the description of the molecular mechanisms necessary for polarisation of Th responses using the murine model of infection with *L. major*, might nevertheless provide critical information to modulate immune responses that may have implications for the design of immunoprophylactic and/or therapeutic intervention.

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