



**Review**

## Cytokine-Producing T Cell Subsets in Human Leishmaniasis

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**Abstract.** *Leishmania* specific Th1/Th2 cells have been identified in humans as well as in mice. There is a correlation between the clinical outcome of the infection and the cytokine response profile. Generally, the production of Th2 cytokines leads to severe infection, whereas the production of Th1 cytokines leads to subclinical or mild infections. In mice, an infection leads to a polarisation of either Th1 or Th2 *Leishmania* antigen specific cells. In contrast, both Th1 and Th2 *Leishmania* antigen specific cells can be identified in humans cured from *L. donovani* infections. Theoretically, Th1 cells and Th2 cells mutually down-regulate each other. However, the presence of antigen specific regulatory T cell subsets may provide an environment that allows the presence of both Th1 and Th2 cells.

**Key words:** human leishmaniasis; cytokines; T cells.

### Leishmaniasis

The symptoms of human leishmaniasis ranges from self-healing, localised ulcers to severe, diffuse, fatal infections involving all lymphoid organs. The disease is distributed in many parts of the world, particularly in Latin America, Africa and Asia, where it affects millions of people. Leishmaniasis has also become an increasing problem in Southern Europe, especially in combination with HIV infection.

The disease is caused by infections with different species of *Leishmania* parasites, which are unicellular protozoan parasites of the family *Trypanosomatidae*. The parasites are hosted by sandflies (*Phlebotomus* and *Lutzomyia*) and mammalian cells – macrophages and monocytes. The present review focuses on two species of *Leishmania* parasites, *L. major* and *L. donovani*. *L. major* is spread throughout North Africa, Middle East, Central Africa and South Asia and causes a lo-

calised form of the disease manifested as one or a few skin lesions that leave a scar. The infections are normally self-healing, although they may lead to persistent lesions. *L. donovani* is found in India, China, East and Central Africa. This species causes a visceral form of disease known as kala-azar. The infection is spread to almost all lymphoid tissues and causes fever enlargement of the spleen and the liver, weight-loss and diarrhoea. Kala-azar is fatal if left untreated. A complication from visceral leishmaniasis, affecting patients who have been successfully drug-cured from visceral leishmaniasis, is post-kala-azar dermal leishmaniasis (PKDL). PKDL patients suffer a severe skin rash, which often affects parts of the body exposed to sunlight. Parasites have been found in the skin of PKDL patients<sup>30, 32</sup> and PKDL has been suggested as a reservoir of infection between epidemics.

The variety of the clinical features of leishmaniasis is large, although infection with *Leishmania* parasites

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may also be subclinical, where individuals are infected without showing any clinical symptoms.

### Experimental Models of Leishmaniasis

Experimental models, such as infections with *L. major* and *L. donovani*, are also extensively studied, especially infections with *L. major* in mice. It is, however, important to keep in mind that infections with the different *Leishmania* species do not give rise to the same clinical features as in humans. Thus, *L. major* causes a progressive and fatal disease in BALB/c mice, whereas these mice can survive infections with *L. donovani*.

Susceptibility to infection with *L. major* in BALB/c mice is associated with an expansion of parasite specific CD4<sup>+</sup> T cells producing IL-4, IL-5, IL-10 and IL-13 (Th2 response). By contrast, resistance to the parasite, e.g. in C57BL/6 mice, results from the development of a polarised response with CD4<sup>+</sup> T cells producing IFN- $\gamma$  and IL-2 (Th1 response)<sup>24, 28</sup>. The outcome of either a protective Th1 response or a fatal Th2 response can be manipulated in these mice early in the infection. Neutralisation of IL-4 can make a susceptible animal resistant<sup>8, 28</sup> and resistant animals which have been manipulated in their inability to produce IFN- $\gamma$  become susceptible to the infection<sup>31</sup>.

As mentioned above, macrophages are both the cells that allow the parasites to multiply and the cells responsible for the killing of the parasite. The intracellular killing is mediated by IFN- $\gamma$  and involves the generation of NO<sup>23</sup>. Furthermore, the effect of IFN- $\gamma$  is synergised by TNF- $\alpha$ , a cytokine that can be produced by macrophages but also by T lymphocytes.

### *Leishmania*-Induced Lymphocyte Response in Unexposed Individuals

Experimental mouse models have provided the basis for similar investigations of the role of cytokines in human leishmaniasis. Generally, it is difficult to compare immune responses in inbred mice to humans. However, results from *in vitro* investigations of human T cells have suggested that features from the *L. major* model in mice may be extrapolated into the human situation<sup>20</sup>.

Measurement of cytokines in culture supernatants by ELISA reveals the quantity of cytokines released to the environment. However, it does not show which cells are responsible for their production. The method

of intracellular staining of cytokines and analysis by flow cytometry has recently been used to identify cellular sources of cytokines in human leishmaniasis. This method was first described in the early nineties<sup>11</sup> and improved when directly conjugated antibodies were introduced<sup>22, 26</sup>.

PBMC from some unexposed individuals can respond to *Leishmania* antigens by the production of cytokines. Since the production of IFN- $\gamma$  may lead to a subclinical infection, it is important to identify the cellular sources of those cytokines. Both natural killer (NK) cells and T cells have been identified as sources of IFN- $\gamma$ <sup>1, 17</sup>. A study from 1997, using intracellular detection of cytokines, revealed that CD3<sup>+</sup> cells were the main source of IFN- $\gamma$ <sup>14</sup>. Using intracellular staining of cytokines and analysis by flow cytometry, we obtained data that support the conclusion that the major part of the T cells responding to the *Leishmania* antigens in such cultures is CD4<sup>+</sup> T cells<sup>15</sup>. Why do T cells from *Leishmania*-naïve individuals respond to *Leishmania* antigens and does this have any relevance? One hypothesis is that cross-recognition of *Leishmania* antigens by CD4<sup>+</sup> T cells may determine whether an individual develops subclinical infections or disease and, subsequently, if the outcome can be predicted before exposure to an infected sandfly. According to this hypothesis, if there is no recognition of the antigens and no production of IFN- $\gamma$ , individuals develop the disease, whereas cross-recognition with subsequent IFN- $\gamma$  production leads to sub-clinical infection.

### Cutaneous Leishmaniasis

Individuals with a history of CL, caused by *L. major*, have acquired immunity to the disease. These individuals mount a strong Th1 *Leishmania*-specific response with high production of IFN- $\gamma$  and low production of IL-4<sup>18, 19</sup>. Furthermore, the clinical spectrum of the disease correlates with the production of these cytokines. Milder presentations are associated with a predominant Th1 response, compared to more severe cases, where Th2 cells also seem to be activated<sup>6</sup>.

Once infection with *L. major* has been established, the severity of the disease seems to be reflected in both the production of IFN- $\gamma$  from CD4<sup>+</sup> T cells as well as other cell types. The production of IFN- $\gamma$  from CD8<sup>+</sup> T,  $\gamma\delta$  T cells and NK cells correlates with the severity of the skin ulcer. One can hypothesize that the parasite infection might lead to a down-regulation of the production of IFN- $\gamma$ , but it is unlikely that a localised parasite infection would lead to a general down-regulation

of IFN- $\gamma$  producing cells in the peripheral blood. More likely, individuals respond differently, either due to environmental factors or genetic differences, and this, in turn, is reflected in the outcome of infection.

When an individual is cured from leishmaniasis, a life-long immunity to the disease is generally developed<sup>25</sup>. However, the response to *Leishmania* antigens is very different in individuals who have suffered from cutaneous leishmaniasis and visceral leishmaniasis. T cells from individuals with a history of cutaneous disease respond by the production of IFN- $\gamma$  and TNF- $\alpha$ , but no or little IL-4 and IL-10. This indicates that a Th1-type memory has been established after natural infection<sup>15, 16</sup>.

### Visceral Leishmaniasis

Kala-azar is associated with a profound lymphopenia and an inability of peripheral T cells to respond to antigenic stimulation *in vitro* by proliferation and cytokine production<sup>2</sup>. This, taken together with the increased levels of certain cytokines<sup>2, 3, 4, 7, 12, 33</sup>, suggests a disease-induced reallocation of *Leishmania* reactive T cells away from the peripheral circulation, as seen in other infectious diseases caused by parasites<sup>9</sup>. Thus, T cells in the lymphoid tissue in patients with acute kala-azar may be very active. We have seen that treatment of leishmaniasis with drugs leads to a rapid emergence of antigen-reactive cells into the peripheral circulation (unpublished data). If these cells have participated in the *in vivo* immune response against the parasite infection, then investigation of such re-emerging cells could prove of great value.

In contrast to cutaneous leishmaniasis, T cells from individuals with a history of visceral leishmaniasis respond to *Leishmania* antigens by the production of IFN- $\gamma$ , IL-4 and IL-10. One hypothesis is that, during the acute disease, T cells in the lymphoid tissues respond to the parasite by the production of IL-4 and IL-10. During treatment, a Th1 response then emerges and leaves the individual with a mixed Th1/Th2 memory response to the parasite. It is generally assumed that IL-4 and IFN- $\gamma$  producing cell populations mutually down-regulate each other. However, *Leishmania* antigen-activated PBMC cultures from individuals with a history of kala-azar often contain both IL-4 and IFN- $\gamma$  producing cells. In such cultures, no cells exist simultaneously producing IFN- $\gamma$  and IL-4, but a cell subset which produces IFN- $\gamma$  and IL-10 has been identified<sup>14</sup>. IL-10/IFN- $\gamma$  producing cells have previously been generated *in vitro* in the presence of IL-10 and named

T-regulatory cells 1 (Tr1)<sup>5</sup>. The IL-10 and IFN- $\gamma$  producing cells found in the *Leishmania* antigen-stimulated cultures might have acquired their cytokine profile during kala-azar when the levels of IL-10 were high and such cells might have provided the environment *in vitro* that allows the presence of both IFN- $\gamma$  and IL-4 producing cells.

PKDL develops in about half of the patients treated for visceral leishmaniasis in Sudan<sup>29, 30</sup>. The lesions mostly heal spontaneously, although, as seen with CL in some patients, the symptoms are severe and persist. PBMC from PKDL patients can also respond to *Leishmania* antigens by the production of IFN- $\gamma$  and IL-10 from CD4<sup>+</sup> T cells<sup>10</sup>.

Generally, *Leishmania*-specific Th1 and Th2 cells are found in both mice and humans following infection, and the clinical outcome is closely correlated to the production of cytokines. However, the situation in humans seems to be more complicated and may involve regulatory T cell subsets.

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