International Journal of Infection
ISSN 1972-6945 / 2024 vol. 8, ISSUE N. 2, May-August

IMMUNOLOGICAL RESPONSE IN LEISHMANIASIS INFECTION

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ABSTRACT

Leishmania is an intracellular parasite that causes the disease leishmaniasis in animals and humans. About 30 species of the genus *Leishmania* can generally infect canids, horses, sheep, cats, rodents, birds and reptiles. Canine leishmaniasis, which is potentially fatal, is present in Europe, Africa, Asia, and America. *Leishmania* parasites complete their life cycle in vertebrate and invertebrate hosts. After infection, the pro-flagellated forms are phagocytised by phagocytic cells and pass to the ovoid flagellated form. The immune response to *Leishmania* infection can be innate or acquired, which defends the host. *L. infantum* infection in humans is mediated by natural killer (NK) cells, monocytes, neutrophils, and cytokines that exert anti-parasitic activity. In leishmaniasis, both Th1 and Th2 lymphocytes are stimulated. Protective immunity against the parasite derives from the development of the Th1 cell response where macrophages play a central role in controlling the infection by linking innate immunity with acquired immunity. Activation of antigen-presenting cells allows activation of T cells against the parasite. Anti-*leishmania* activity can be carried out by tumor necrosis factor (TNF), IL-6, and by costimulatory cells B7-1 (CD80) and B7-2 (CD86).

KEYWORDS: *Leishmania, leishmaniasis, infection, immunity, parasite*

INTRODUCTION

Leishmania is a protozoa belonging to the Trypanosomatidae family (1). It is an intracellular parasite which causes the disease leishmaniasis in animals and humans. The approximately 30 species of the genus *Leishmania* can infect humans, dogs, wild species of the canidae family (e.g. foxes, jackals), horses, sheep, cats, various species of rodents, birds, and reptiles (2-4) and are found in almost country around the world (5). Canine leishmaniasis is caused by the species *Leishmania infantum* or the genetically identical species *L. chagasi* (6) and is a potentially fatal enzootic disease in regions of Europe, Africa, Asia, and America (7).

Leishmania parasites are biphasic protozoa that alternate between proflagellate and α-flagellate forms and complete their life cycle in vertebrate and invertebrate hosts (8,9). Female midges of the genus *Phlebotomus sp.* are mentioned as intermediate hosts in Europe, Asia, and Africa and genus *Lutzomyia sp.* are intermediate hosts in America (5,8).

The ovoid flagellar forms (2-5 μm x 1.5-2 μm) are characterized by the presence of rodlike kinetoplasts, DNA-rich mitochondrion, and parasitize the macrophage cells of the skin, mucous membranes, and viscera of the definitive hosts (10). Flagellar forms are consumed by flies during blood sucking and are released into the posterior part of the mesentery where they are transformed into motile proflagellar forms and multiply rapidly by binary fission, attaching to several sites

Received: 04 March, 2024
Accepted: 08 July, 2024

1972-6945 (2024)

of the insect's mesentery wall via lipophosphoglycans (11). They then transform into infective metacyclic proflagellate forms, which migrate to the esophageal valve of the thoracic mesentery, pharynx, and mouthparts of the fly. The parasite is transmitted to vertebrate hosts during sandfly molt, primarily by reduction from the thoracic mesenchyme to the host's skin. After inoculation into the skin of the definitive host, the proflagellate forms are phagocytosed by antigen-presenting Langerhans cells (in the epidermis), dendritic cells (in the dermis) and macrophage cells, where they transition to the ovoid flagellate form (12-14). In turn, the female phlebotomes become infected when they consume female blood from infected dogs, and they acquire the astigmatism forms. In Greece, 12 species of sandflies have been described, showing intense activity from the beginning of May to the end of November (13).

DISCUSSION

In Greece, Portugal and the other Mediterranean countries, where the species *L. infantum*, *L. tropica* and *L. major* have been isolated, leishmaniasis has an enzootic character (15,16). In seroepizootic studies carried out in Italy, Spain, France, and Portugal, the number of infected dogs is estimated to be around 2.5 million (17,18).

Estimating the number of infected dogs presents problems due to the existence of asymptomatic carriers, the long incubation period of the disease (which can reach up to 7 years), and the inability of serological tests to detect infected but non-seropositive animals (18-20). Today, however, with the widespread use of molecular methods to detect the genetic material of the protozoan, it appears that the frequency of infection is much higher than the frequency of the symptomatic form of the disease (18).

The recent increase in the occurrence of cases in non-enzootic areas such as the Netherlands, Germany, and the United Kingdom is mainly attributed to the infection of dogs during their stay in enzootic areas as well as the importation of dogs from enzootic areas in Southern Europe, while the existence of autochthonous foci of L. infantum in Northern and Central Europe cannot be ruled out (21,22).

With regard to public health, it is now widely accepted that dogs are the main reservoir of *Leishmania* in nature and are directly involved in the epidemiology of some forms of human leishmaniasis in various regions of the world (5,7,20). The presence of infected dogs in populated areas is clearly related to the transmission of the disease to humans. However, ownership of an infected dog does not seem to significantly increase the probability of infection of owners in areas where the disease is endemic (20).

Immune responses

Clinical observations argue for the importance of the host immune response in control of leishmaniasis. The immune response is divided into innate and acquired immunity.

The innate immune response to *L. infantum* infection is mediated by natural killer (NK) cells and cytokines, and phagocytosis is mediated by monocytes and neutrophil polymorphonuclears. Blood phagocytes (monocytes and granulocytes) and tissue macrophages exert antiparasitic activity (26).

In addition to natural immunity, acquired immunity also participates in *L. infantum* infections in dogs. Cellular immunity plays a decisive role in the development of infection. The onset of leishmaniasis symptoms is associated with suppression of cellular immunity and humoral stimulation with the production of antibodies (27).

In protozoan diseases, the immune response depends on both the parasite itself and the host (23). Protozoan parasites develop survival mechanisms and ways to escape the host's immune response. The host, depending on the immune response it develops and its immune capacity, may be susceptible or resistant to infection (24,25).

Cellular immune response

Leishmania parasites stimulate both Th1 and Th2 lymphocytes in infection of dogs (7). Previous studies in experimental models have shown that symptoms, and their degree of intensity, depend on the type of immune response and, specifically, on the selective activation of the Th1 or Th2 responses (28). Today, it is widely accepted that protective immunity against the parasite results from the development of a strong Th1 cell response. Conversely, disease may occur if the Th2 cell response predominates (7).

Macrophages play a central role in controlling infection by linking innate and acquired immunity (29). In addition to their phagocytic capacity, macrophages can also process and present parasite antigens with simultaneous expression of costimulatory molecules, such as B7-1 (CD80) and B7-2 (CD86) (30). Their antigen-presenting abilities and concomitant secretion of cytokines, such as IL-1, tumor necrosis factor (TNF), IL-12, and IL-6, enable the activation of specific CD4+ and CD8+ T lymphocytes (29).

In addition to the activation of T lymphocytes, the above cytokines act protectively by stimulating the anti-*leishmania* activity of macrophages, primarily through the production of oxygen free radicals and nitric oxide (NO) (18).

NO is produced by activated macrophages and is mostly responsible for the intracellular death of flagellar forms of *Leishmania* through apoptosis (31). The production of NO and its anti-*leishmania* activity was confirmed after a series of *in vitro* studies, where macrophage cell lines from infected dogs were incubated with cytokines, including IL-2, TNF, and interferon-gamma (IFN-γ) (32). Similar findings were also found in a study where macrophage cell lines from healthy dogs were incubated with recombinant human IFN-γ after infection with proflagellate forms of the parasite (33).

The production of NO is catalyzed by the enzyme iNOS, whose activity is regulated at the level of transcription (34). Activation of appropriate transcription factors is regulated by the ERK1/2, p38, and JNK kinases message transduction pathways. Transcription factors, such as NF-κB, move to the cell nucleus after activation, where they bind to the promoters of various genes, regulating the expression of pro-inflammatory cytokines (e.g., TNF) and enzymes (e.g., iNOS) (35).

The T lymphocyte response to intracellular antigens consists of a series of sequential phases that begin with recognition of the antigen by naïve lymphocytes, activation and expansion of antigen-specific clones by proliferation, and differentiation of some of the progeny cells of these in reactive and memory cells (29). Differentiation of CD4+ T cells into Th1 or Th2 cells is a determinant of host resistance or susceptibility to parasite infection (Fig.1). However, to date, the exact molecular mechanisms that trigger the Th1 or Th2 immune responses have not been elucidated (36). Two hypotheses have emerged from *in vivo* human studies to interpret host resistance or susceptibility to *L. infantum* infection (26).

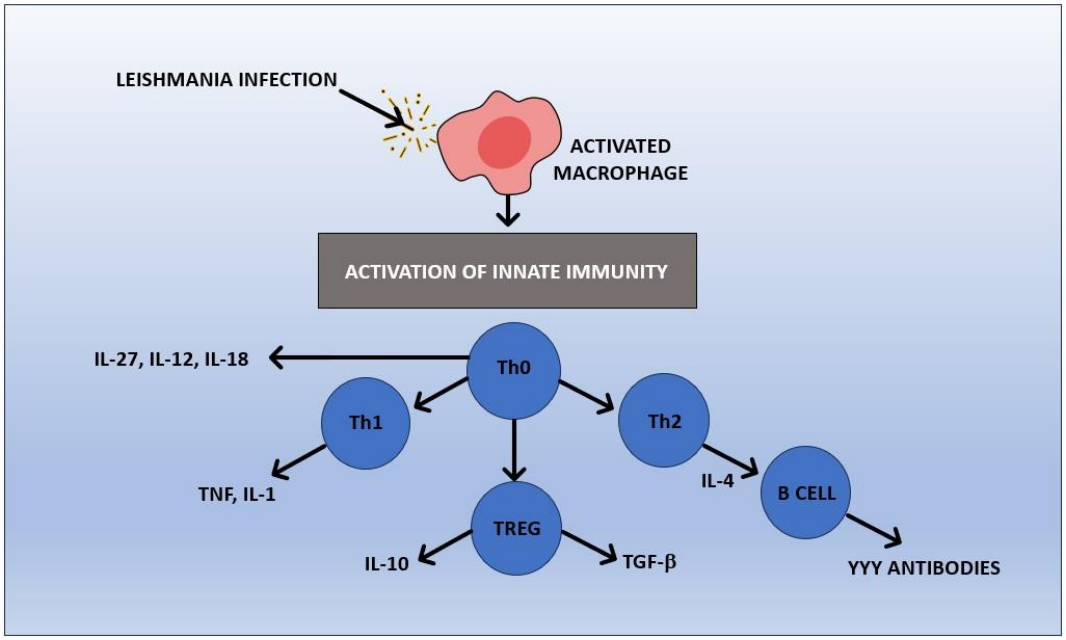

Fig. 1. *Schematic illustration of the cellular immune response in Leishmaniasis, showing the differentiation of Th0 to Th1 or Th2 cells and the corresponding cellular immune responses that are induced. Once Leishmania enters an organism, it induces the activation of macrophages. Th0 (T helper zero) cells are activated, followed by the release of the cytokines IL-27, IL-12, and IL-18. Th0 cells can mature into Th1 cells (with the production of inflammatory cytokines TNF and IL-1), Th2 cells (with the generation of IL-4 that helps B cells to produce antibodies), or Treg cells (with the production of anti-inflammatory cytokines IL-10 and TGF-beta).*

The first hypothesis focuses on CD4+ T lymphocytes during the first days after infection. Analysis of cytokine production by CD4+ T lymphocytes showed a dramatic burst of IL-4 production, which on the fourth day after infection, gradually decreased in resistant patients, while remaining at high levels in susceptible patients. The above findings indicate that susceptible patients produce an excess of IL-4 and are highly sensitive to its action (26).

The second hypothesis focuses on the action of NK cells. In resistant patients, a strong NK cell response is observed after infection. Activated NK cells produce IFN-γ, which leads to a Th1 cell response resulting in host resistance. Infection with the parasite of BALB/c mice, which have a primary reduced number of NK cells, results in the manifestation of the disease with a clear predominance of the Th2 humoral immune response (26).

In 30-50% of dogs living in the enzootic areas of the disease, it has been established that their basic immune response is of the cellular type, which is also indicated by a positive intradermal reaction to an acquired protein of the parasite (37).

This delayed-type hypersensitivity (DTH) reaction is observed in disease-resistant dogs exposed to the parasite, while it is absent in those presenting with severe clinical presentation (20).

The ability to maintain an effective anti-*leishmania* acquired cellular immune response entails arresting parasitemia and parasite multiplication in target organs (38). The phenomenon is observed in resistant dogs, where infection with *L. infantum* remains asymptomatic or manifests with mild and self-limiting symptoms, while these responses decrease as the disease progresses in susceptible dogs (39). Blood parasite load and the acquired cellular immune response were shown to be inversely related during a long-term study of experimentally infected dogs (38).

The cellular basis and developmental mechanisms of T cell irresponsiveness in canine leishmaniasis are not fully understood. The non-response of cellular immunity in advanced stages of the disease may be due to:

(a) the reduced expression of co-stimulatory molecules by infected macrophages;

(b) the reduction of the number of peripheral CD4+ T lymphocytes (as recorded by flow cytometry studies); and

(c) the reduced production or clearance of IL-2 and IFN-γ by peripheral blood monocytes *in vitro* (27).

In vulnerable dogs, Th2 lymphocytes are activated and, through the release of interleukins IL-4, IL-5, IL-10 and IL-13, favor the uncontrolled proliferation of the parasite and the stimulation of humoral immunity (40) (Fig.1). IL-2, IL-4, IL-5, and IL-13 induce the proliferation of B lymphocytes and promote the production of IgG1, IgG4, IgE, and IgA (18).

Finally, the cytokines of the Th2 response act antagonistically with IFN-γ and suppress the activities of Th1 lymphocytes, NK cells, and monocytes/macrophages. It should be noted that the activation of the above mechanisms has been verified in mice and humans (41), while in symptomatic dogs, it is indirectly inferred from the reduced production of IFN-γ and TNF and the number of CD4+ and/or CD8+ lymphocytes (42).

CD8+ cytotoxic T lymphocytes have been shown to be involved in dog resistance to leishmaniasis, although studies regarding this cell population remain limited (43). Activated macrophages promote the activation of CD8+ T lymphocytes and IL-2 produced by Th1 cells, which act as autocrine growth factors and, together with IFN-γ, activate the proliferation and differentiation of cytotoxic CD8+ T lymphocytes (27).

CD8+ T lymphocytes have been detected in experimentally infected asymptomatic dogs (but not in symptomatic ones), suggesting that they represent an additional effective resistance mechanism (44). The participation of these lymphocytes in leishmaniosis is also indicated by the fact that in dogs naturally infected with *L. infantum*, a reduction of the specific cell population is observed as the infection progresses, while its restoration takes place after the administration of treatment (45).

Other studies attributed an additional role to CD8+ T lymphocytes, noting that they are able to stimulate an adequate Th1 response with production of IFN-γ and TNF and the ability to activate infected macrophages (27,46). Today, elevated levels of CD8+ T lymphocytes are the main phenotypic feature of the asymptomatic form of the disease (43,44).

In addition to the differences in the absolute numbers of lymphocytes, differences in their activation levels were also recorded. High levels of circulating activated leukocytes were observed in asymptomatic dogs, as evidenced by high expression of major histocompatibility complex (MHC) class II molecules, CD45RB and CD45RA. In contrast, symptomatic dogs showed reduced expression of MHC class II molecules, reduced antigen-presenting capacity, and reduced numbers of antigen-presenting B lymphocytes (43).

Subclinical infection is not necessarily permanent and, in cases of a compromised immune system due to immunosuppression or coinfections, it may progress to clinical infection (47). The humoral immune response is not protective and indicates failure to control the infection (7,27,38,41).

CONCLUSIONS

Leishmaniasis is a disease caused by the *Lieshmania* parasite that is transmitted by animals such as canids. *Leishmania* infection triggers an immune response that can be innate or acquired. *L. infantum* infection in humans is mediated primarily by innate immune cells such as NK cells, monocytes, and neutrophils. These cells react against the parasite to protect the body from *Leishmania* infection.

Conflict of interest

The authors declare that they have no conflict of interest.

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