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Immune response to *Toxoplasma gondii*

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Summary. - Oral-route infection with *Toxoplasma gondii* sporozoites or tachyzoites leads to the rapid spread of quick-replicating cytolytic tachyzoites throughout the whole body. *Toxoplasma* easily crosses the blood-retina, encephalic and placental barriers. The acute phase of this infection lasts for less than around ten days. The parasite causes a very strong type-1 response focused on the interferon-gamma secreted by the T lymphocytes. This immune response limits the tissue extension of the parasite, ensuring the survival of the host, but, paradoxically, also aiding the survival of the parasite by converting it into a bradyzoite, an intracellular quiescent resistant form persisting in the muscle and brain tissues.

Key words: Toxoplasma, humoral immune response, cytochine, pathology.

Riassunto (Risposta immune a *Toxoplasma gondii*). - La via orale d'infezione con sporozoiti o tachizoiti di *Toxoplasma gondii* porta alla rapida comparsa di tachizoiti citolitici velocemente replicantesi in tutto il corpo. *Toxoplasma* facilmente attraversa le barriere della retina, encefalo e placenta. La fase di questa infezione perdura per meno di circa dieci giorni. Il parassita causa una forte risposta di tipo I concentrata sull'interferon-gamma prodotto secreto dai linfociti T. Questa risposta immune limita il propagarsi del parassita nei tessuti, assicurando la sopravvivenza dell'ospite, ma, paradossalmente, anche aiutando la sopravvivenza del parassita convertendolo in bradizoita, una forma intracellulare quiesciente che persiste nei tessuti muscolare e cerebrale.

Parole chiave: Toxoplasma, risposta immune umorale, citochine, patologia.

Introduction

The immune response to *Toxoplasma gondii* infection is individual, complex and compartmented. This individual variation can be explained by the high level of heterogeneity in genetic background. In addition, *Toxoplasma* has the capacity to spread in all the tissues and each tissue compartment has its own specific immune response, particularly in the central nervous system and in the placenta. An additional degree of complexity is achieved due to the possibility of recurrent infection with strains of *Toxoplasma* with variable virulence.

Most of the data mentioned in this review of the literature have been obtained from studies in animals and, in particular, in mice. In essence, data acquired in animals cannot be directly transposed to humans. However, in the specific case of the mouse model, this model presents a vast spectrum of immune responses to *Toxoplasma* related to controlled variation of the genetic background of congenic laboratory animals [1]. This choice of murine model makes it possible to approach certain specific aspects of the pathophysiology of human *Toxoplasma* as closely as possible.

In general, the immune response of a non-immunodepressed host developed in the course of *Toxoplasma* infection, leads to the acquisition of protective immunity for the foetus in the event of re-infection. However, the parasite persists in its bradyzoite form, inside the intracellular cysts. The periodic rupture of these cysts is thought to be the origin of maintained immunity against *Toxoplasma*. In the event of immune deficiency and, in particular, AIDS, the bradyzoites released following rupture of a cyst are converted into tachyzoites, the proliferation of which is not effectively controlled by the immune response of the host, leading to severe brain damage [2, 3]. Cellular immunity is therefore the key component of the host's immune reaction in the event of attack by *Toxoplasma* [4, 5]. The macrophages, T lymphocytes T (TL) and "natural killer" (NK) cells, on the one hand, and the cytokines, on the other, are the major elements involved in immune response. Antibodies play a minor role but remain the essential means for diagnosing toxoplasmosis in humans.

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The natural or non-specific immune response

T. gondii is capable of triggering the non-specific activation of macrophages and NK cells [6] along with other haematopoietic and non-haematopoietic cells. This activation is intended to limit parasite proliferation due to its direct or indirect cytotoxic action and to trigger a specific immune response due to the presentation of *Toxoplasma* antigens. This non-specific response begins immediately following the first contact between the parasite and the host. It peaks at the end of the first week, and then slowly reduces until it finally disappears two weeks after the start of infection.

In mice, the activation of macrophages by IFN-γ in the presence of co-signals, such as LPS or TNF-α, is necessary to trigger the cytotoxic activity of the macrophages against *T. gondii* [7]. The inhibition of Toxoplasma replication or its destruction are the result of various effector mechanisms: a) oxidative mechanisms [8, 9]; b) non-oxidative mechanisms, represented mainly by the production of nitrogen monoxide (NO) by macrophages activated by IFN-γ [10-12], with NO also involved during the chronic phase due to its inhibition of intracerebral parasite proliferation [13]; c) non-oxygen-dependent mechanisms may also be toxoplasmicidal, such as the induction by IFN-γ of indoleamine 2,3-dioxygenase, which degrades the tryptophan required for growth of the parasite [14]. But this process is the subject of controversy [15].

The role of NK cells has been explored *in vivo* in a model of immuno-depressed SCID mice (severe combined immunodeficiency). In this model, resistance to *Toxoplasma* infection is related to the production of IFN-γ, which - in the absence of functioning CD4+ and CD8+ cells - originates from NK cells [16] [17]. This NK activity is dependent (i) on soluble factors secreted by the activated macrophages: IL-12 and TNF-α [18] [19] and (ii) on signalling pathways such as STAT4 [20].

During the early phase of the infection, it is through the combined and synergetic action of the NK cells and the macrophages, activated by IFN-γ, that most non-specific or natural resistance not restricted to the MHC is exerted. At this stage, monocyte macrophage lineage cells differentiate into antigen-presenting cells (APC).

Other cells are also involved in this non-specific resistance. Human γ-δ TL expresses a cytotoxic activity *in vitro*, not restricted to the MCH, against cells infected with *T. gondii*. They also secrete IFN-γ, IL-2 and TNF-α in the presence of stimulation by the parasite [21]. An increase in this cell population has been found in humans in the course of *Toxoplasma* infection, but also in the spleens of mice infected by the intraperitoneal route [22-24]. Due to their preferential location in the intestines, these cells could represent an important component of the early immune response that occurs following infection by the oral route, which is the commonest method of contamination in humans [25]. However, the role of γ-δ TL appears to be minor in mice [26]. Platelets also appear to be able to exert a cytotoxic activity against *T. gondii*, independently of specific antibodies [27, 28]. Neutrophils and, very probably, eosinophils, and mast cells rapidly intervene at the site of infection and are involved in setting up a non-specific early immune response via the production of IL-12 and various pro-inflammatory factors [29-32]. And, finally, non-haematopoietic cells (fibroblasts, epithelial or endothelial cells, etc.) are also capable of reducing parasite proliferation according to mechanisms dependent on Iron, iNOs, IFN-γ, and TNF-α [33, 34].

Specific acquired immune response

The non-specific immune response has led to differentiation of macrophages and BL into APC. The effector cells are stimulated by dendritic cells presenting the antigen to TL TCR. However, this mechanism requires a close interaction between the APC and the TL thanks to the CD40-CD40L system [35, 36]. Infection of the dendritic cells by live *Toxoplasma* exclusively leads to activation of CD40 in humans [37].

These effector cells, which are involved in resistance to *Toxoplasma* infection, then exert their function via a cytotoxic activity and/or the secretion of cytokines involved in the regulation of immune response [38].

CD4+ and CD8+ TL are the main players involved in resistance of the host to *Toxoplasma* infection [39] [40]. In mice, mature CD4+ TL are divided into two sub-populations: Th1 and Th2. This distinction is based on the list of cytokines secreted following stimulation, as reported by Mosmann in 1986. The type-1 cells produce IL-2 and IFN-γ while the type-2 cells produce IL-4, IL-5, IL-6 and IL-10. CD4+ TL are required for the development of resistance during the early phase of the infection [41], and for immunity during vaccination [40, 42]. This resistance is closely related to a type-1 response promoted by the IFN-γ and IL-12 produced following activation of NK cells and macrophages [43, 44, 18]. However, it has been shown that control of *Toxoplasma* infection is the result of a synergetic action between CD4+ TL and CD8+ TL [45, 40].

The CD8+ TL, activated more especially by the surface proteins of the parasite [46], appear to be essential in resistance during the active phase of *Toxoplasma* infection, both in humans and in mice, and enable a protective immunity to be passed on [47, 48].

The CD8+ TL, activated by the IL-2 secreted by the CD4+ TL, exert a cytotoxic activity against tachyzoites or cells infected with *T. gondii* [49, 47]. This activity, exerted by IFN-γ, is obviously restricted by class-I MHC and helps to provide the host with resistance during the chronic phase of *Toxoplasma* infection [50, 44, 51].

The persistence of the memory of TL in toxoplasmosis in humans is an established fact. Indeed, in humans, a primo-infection protects the foetus in the event of subsequent re-infection. In addition, the anti-*Toxoplasma* antibody remains detectable throughout the lifetime of the host. It is probable that the persistence of the memory of TL is guaranteed by the regular rupture of intracellular cysts and also by recurrent food infections. The persistence of the memory of TL is ensured by intracellular signalling mechanisms employing NF-k B(2) [52] following activation by the surface proteins or dense granule proteins of the *Toxoplasma* [53]. It is recognised that the activation of CD28 lymphocyte receptors, dendritic B7-1 (CD80) and B7-2 receptors is essential for the acquisition of a good lymphocyte memory [54].

Cytokines

Cytokines are soluble mediators secreted by the cells without any specificity for antigens and which exert their biological action at very low concentrations. They act on numerous cells. Their action is essentially local and leads to a modification in cell behaviour due to paracrine and autocrine effects. In the event of toxoplasmosis, they can be divided into 2 main types - protective and regulatory cytokines - without taking into account their strict classification into type-1 or type-2 cytokines. There is a delicate balance between protection and regulation, and this can be accentuated in an exaggerated manner towards both type-1 immune diseases and type-2 immunosuppression, depending on the host (genetic background, iatrogenic immunosuppression or otherwise) or the virulence of *Toxoplasma* strains.

Protective cytokines

Interferon γ (IFN-γ) has numerous biological activities, including: activation of macrophages and NK cells, induction of MHC class-II antigens and inhibition of type-2 cell response. NK cells and TL (CD4+ and CD8+) are the main sources of IFN-γ. Identified back in 1966, in the peritoneal fluid and serum of mice infected with the virulent RH strain [55], IFN-γ was the first cytokine implicated in resistance to *T. gondii* and remains the keystone of protective immunity to *Toxoplasma* [56, 44]. IFN-γ is produced during *Toxoplasma* infection both in sensitive mice and in mice resistant to infection [2]. The production of IFN-γ is also found in humans in acute toxoplasmosis and in newborn babies infected during pregnancy, with a correlation between the degree of foetal infection and the quantity of IFN-γ secreted [57]. In mice, the secretion of INF-γ increases the phagocyte activity of macrophages and the cytotoxic activity of CD8+ TL [58]. However, IFN-γ triggers the conversion of tachyzoites into bradyzoites [59, 60] [61] at the same time preventing their rupture [44, 2]. A high level of IFN-γ production is strongly correlated with virulent type-1 strains and increased apoptosis [62] and also with intestinal immunopathological phenomena in C57BL/6 sensitive mice [63, 64].

Interleukin 12 (IL-12), which is secreted by the macrophages and the dendritic cells during antigen stimulation, appears to play a major anti-*Toxoplasma* role during the acute phase of the infection. Indeed, it activates the production of IFN-γ by NK cells and CD4+, CD8+ TL [65]. The administration of IL-12 combined with the recombinant *T. gondii* SAG1 (surface antigen 1) surface protein directs the immune response towards a predominantly type-1 profile, associated with high IFN-γ production. This directing of the immune response is linked to a reduction in cerebral parasite load [66]. IL-12 is also essential during the chronic phase of the infection, when it is responsible for maintaining a long-term immune response [67]. The positive regulation of IL-12 is obtained via CCR5-type receptors [68] whereas negative regulation is obtained via lipoxins A [69].

TFN-α is produced by monocyte macrophages, TL and basophil mastocytes. It exerts an early protective effect by increasing the microbicidal capacities of the macrophages and inducing the secretion of IFN-γ by the NK cell. A pyrogenic factor, TNF-α is liable to induce the secretion of acute inflammatory phase proteins via the production of IL-6. In toxoplasmosis, TNF-α would appear to be essential for macrophage activation and inhibition of parasite replication, but this action can only be exerted in synergy with IFN-γ. This protective action is exerted in mice in both the acute and chronic phase of the disease [70-72]. In addition TNF-α - like IL-12, another monocyte macrophage product - stimulates the production of IFN-γ by NK cells [16, 73], which play a crucial role in the early non-specific response during toxoplasmosis. However, the role of TNF-α in toxoplasmosis is still debated. Some authors report a link between TNF-α and fatal infection in mice and with a harmful cerebral and hepatic action [74-76]. TNF-α may aid the intracerebral dissemination of *T. gondii* in mice [77] and may be increased in toxoplasmic chorioretinitis during primo-infection in humans [78].

Interleukin 6 (IL-6) is produced by a large number of cells, including monocyte macrophages, endothelial cells, fibroblasts, myelomatous and neoplastic cells. The main mediator responsible for hepatocytic production of acute inflammatory phase proteins, it exerts a synergetic action with IL-1, TNF-α and glucocorticoids. IL-6 is therefore a pyrogenic factor and a remarkable stress marker. IL-6 increases the cytotoxic activity of NK cells and later induces differentiation of BL into antibody secreting cells and differentiation of cytotoxic TL. In murine toxoplasmosis, a gradual increase in serum IL-6 is correlated with clinical signs [75]. The administration of an anti-IL-6 monoclonal antibody in a model of murine toxoplasmic encephalitis reduces the inflammatory lesions and number of cysts in the brains of these mice [79]. In ocular toxoplasmosis in IL-6 -/- mice, IL-6 has a protec-tive role [80]. However, other reports are contra-dictory. According to Beaman, IL-6 appears to promote the intracellular multiplication of *T. gondii* in mice [81] whereas for other authors, human monocyte macrophages or cells derived from a human astrocytoma do not secrete IL-6 *in vitro* in response to toxoplasma infection [82-84].

Interleukin 5 (IL-5) is produced by numerous cells (TL, mastocytes, eosinophils). IL-5 triggers the growth, differentiation, activation and chemotaxis of eosinophils. It is surprising to observe that in toxoplasmosis, this cytokine is capable of increasing the production of IL-12 and of inducing a certain protection in mice against *Toxoplasma* infection [85] although other authors attribute a pathogenic role to it, through an increase in intestinal necrosis [86]. The presence of eosinophils in human congenital toxoplasmosis is probably related to the production of IL-5 [87, 88].

Interleukin 15 (IL-15) is a pleiotropic cytokine secreted by various cells, including macrophages [89]. It also appears to play an important role, inducing the maturation of NK cells and the proliferation of CD8+ TL [90]. It prolongs the activity of these cytotoxic CD8+ TL [91] and increases the production of IFN-γ in experimentally-induced infection with *T. gondii* (Lee *et al*., 1999).

Interleukin 18 (IL-18) is another pleiotropic cytokine produced in a non-specific manner during an inflammatory syndrome. It has the capacity to increase the activity of NK cells in experimentally-induced toxoplasmosis and requires a STAT 4-type transcription factor [19, 20].

Interleukin 2 (IL-2) is produced exclusively by the CD4+ TL. In murine toxoplasmosis models, IL-2 has been shown to be protective. The administration of recombinant IL-2 in mice sensitive to *Toxoplasma* infection leads to an increase in the survival of the animals and a reduction in the number of cysts present in the brain. A parallel increase in lytic activity of the macrophages against *Toxoplasma* and of NK cell activity is also observed [43, 47, 92].

Regulatory cytokines

Interleukin 4 (IL-4) is secreted by a small number of cells and, more specifically, by type-2 CD4+ TL. Basophils, mastocytes and certain CD8+ TL can also be a source of IL-4. A factor for the activation and differentiation of TL and B lymphocytes (BL), it increases the expression of class-II MHC antigens and triggers IgE isotype switching. IL-4 alone does not appear to influence the intracellular growth of *Toxoplasma in vitro* [93]. However, *in vivo* in mice, endogenous IL-4 appears to play an important role in resistance to *Toxoplasma* infection [94] but it is believed that it may play an immunosuppressant role promoting the passage of *Toxoplasma* through the placenta in mice [95, 96].

Interleukin 10 (IL10) is secreted by type-2 CD4+ TL, macrophages and certain BL. IL-10 inhibits the proliferation of type-Th1 CD4+ TL, along with the secretion of cytokines by these same cells. It also inhibits the production of nitrate and oxygenated derivatives and of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) by monocyte macrophages. In toxoplasmosis, the *in vivo* administration of an anti-IL-10 monoclonal antibody to SCID mice delays the death of the animals following Toxoplasma infection [97]. *In vitro*, recombinant IL-10 has immunosuppressant properties on the proliferation of spleen cells taken from mice infected with *T. gondii* [98] and inhibits the capacity of murine macrophages, activated by IFN-γ, to destroy *T. gondii* [99]. IL-10 is therefore necessary for the negative regulation of a type-1 intestinal response that may be harmful in C57BL/6 susceptible mice. IL-10 counters the harmful effect of an exaggerated type-1 inflammatory response based on the high production of TFN-α, IFN-γ and NO associated with the intestinal proliferation of *T. gondii* [100, 64, 101].

Transforming growth factor-β (TGF-β) is well-known for its immunosuppressant action on leukocyte cell lines. It is considered to be an antagonist of TNF-α, IFN-γ, TNF-β and IL-2 [102] [103]. The anti-inflammatory action of this cytokine makes it possible to control the development of immunopathological phenomena related to a type-1 immune response in the intestines [104] or the brain [105]. However, TGF-β increases replication of the *Toxoplasma* on cultured retinal cells, suggesting that this cytokine may be involved in immunopathological phenomena [106].

Chemokines

One of the functions of chemokines is to induce chemotaxis of NK cells, leukocytes and TL. Their presence has been observed during toxoplasmosis. The production of MuMig, Crg-2 or IP-10 chemokines, produced by the hepatocytes and NK cells, attracts the TL to infected sites and supports the observations made relative to the involvement of these cells in tissue damage [107, 108].

Humoral immune response

IgM are the first antibodies to appear. They are detected in the peritoneal fluid of mice, at the surface of the *Toxoplasma*, from the second day following infection. However, serum IgM only appears at the end of the first week following infection. These immunoglobulins are the best activators of the complement system. In addition, due to their structure, they enable excellent agglutination and have a high level of cytotoxicity. This phenomenon is used especially in serological diagnosis techniques. Their persistence is subject to a high level of individual variation and can be as much as a year in most cases, thanks to the use of increasingly sensitive detection techniques. The major target antigens of these IgM are the surface proteins of the parasite. "Natural" IgM are often involved in the serological diagnosis of toxoplasmosis. Examples of "natural" IgM are anti-A and anti-B isohemaglutinins, IgM directed against typhoid antigen O (endotoxin) or syphilis antigen WR. The presence of intracytoplasmic parasite substances, such as HSP, actin, myosin, tubulin or calmodulin, may explain the false positives observed during serological diagnosis of parasite infections. Although real, as yet no satisfactory explanation has been given for the existence of natural IgM during toxoplasmosis.

IgG are the second immunoglobulins to appear in toxoplasmosis. There are four sub-classes, which appear in unequal proportions during toxoplasmosis. IgG1, G2 and G3 are thought to be predominant. They also enable antibody-dependent cytotoxicity (ADCC) or opsonization, thanks to Fc receptors existing on the monocyte macrophages and the polynuclear cells or to cytolysis mediated by the complement or by an NK cell. They play a role in protection of the foetus because they are capable of crossing the placenta. The main target antigens of IgG are the surface antigens of the parasite.

IgA are observed in two forms: mucosal IgA appearing in the mucous secretions and serum IgA. In toxoplasmosis, both types of IgA are found both in the digestive tract and serum in animals and humans. In acquired toxoplasmosis, the appearance of IgA is not systematic, but the onset kinetics appear to be short, with negativation obtained after 1 year. In immunodepressed subjects, IgA is thought to be an early marker in 50% of cases [109]. In congenital toxoplasmosis, the detection of IgA is particularly valuable, since these can be detected in the absence of IgM. IgA (like IgM) do not cross the placenta and are actively involved in the diagnosis of congenital toxoplasmosis [110].

Few studies have been conducted on IgE. Their appearance in acute or congenital toxoplasmosis is random. The presence of this isotype is correlated with the onset of complications, such as adenopathies, chorioretinitis, and *Toxoplasma* reactivations in immunodepressed subjects [111]. However, analysis of this isotype is only useful if it is combined with analysis of IgM and IgA.

In conclusion, antibodies are the body's first line of defence. They act on the extracellular tachyzoites released following lysis of infected cells. They limit multiplication of the *Toxoplasma*, by lysing the parasites in the presence of the complement. They are also active via opsonization or via an increase in phagocytosis by the macrophages. Hence, antibodies are shown to be indispensable for effective vaccination in mice [112]. But it has also been demonstrated that a strong antibody response appears to promote the formation of intra-cerebral cysts [112].

Mechanisms of parasite evasion

These are of several types:

- the role of the parasitophorous vacuole: the presence of the parasite in a parasitophorous vacuole enables it to escape the humoral immune defences (AC, enzymes, proteolytics and complement). Modification of the wall structure by ROP and GRA proteins prevents trans-membrane exchange and fusion of the lysosomal vacuoles [113];
- the various parasite stages ensure renewal of the antigens, requiring an adjustment of the host's immunological elimination process. The change in tachyzoite stage is promoted by the presence of NO and is manifested by major isoenzymatic variations [114];
- molecular mimesis: toxoplasma shares some epitopes with its host, including cerebral epitopes [115]. This could explain the capacity of *Toxoplasma* to remain undetectable in the brain tissues but poses the problem of the onset of neurological diseases, such as schizophrenia, following *Toxoplasma* infection [116];
- immunosuppression: this more specifically involves immuno-modulation of immune response. Under the influence of IFN-γ, NO is capable of reducing lymphocyte proliferation [117]. The same is true for IL-10 [98]. This is directly under the control of *Toxoplasma* proteins, such as SAG1 and microneme proteins (MIC) [118]. But this suppression is only

exerted on the splenic and mesenteric lymphocytes [119]. During the course of *Toxoplasma* infection, the production of IL-12 by dendritic cells is reduced, whereas the production of IL-10 is increased [120]. In some ways, these immunosuppressant mechanisms prevent the development of type-1 immunopathological phenomena [121]. They ensure the survival of the host, but also that of the parasite;

- apoptosis: the parasite induces apoptosis of CD4+ cells in the acute phase [122], thus partly contributing to the immunosuppression observed during the acute phase of the disease. However, infection of a cell by *Toxoplasma* inhibits its apoptotic capacities and guarantees the survival of the infected cell by preventing its lysis via the Fas system [123].

Conclusions

Control of a *Toxoplasma* infection is complex and depends on the genetic background of the host, his immune status and also parasite factors, including virulence. Most of the data in this review of the literature was obtained from experimental murine models. However one may view these data sceptically when comparing them to humans, the murine model is a source of advances enabling us to focus immunological exploration in humans on certain areas. Moreover, on the basis of genetic background - and here we include transgenic mice - mice make it possible to explore specific compartments, such as the intestines [64], the placenta [95] or the eye [80]. The existence of *Toxoplasma* strains in which the virulence (and, by extension, the immune response) varies in animals does not appear to have significantly influenced human resistance to *Toxoplasma*, except in the event of contact with wild South American strains, in which immunopathological phenomena are observed [124, 125]. Toxoplasmosis is therefore a disease whose clinical expression is mild or even non-existent, making exploration of its pathophysiology difficult. Thus, if we want to further our knowledge in the field of *Toxoplasma* immunology, we must continue to develop experimental models close to humans (transgenic mice, rats, guinea-pigs, and sheep).

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