Functional exhaustion of T lymphocytes in chronic toxoplasmosis

Henryka Długońska, Marcin M. Grzybowski

Department of Immunoparasitology, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Corresponding author: Henryka Długońska; e-mail: hdlugo@biol.uni.lodz.pl

ABSTRACT. One of the most characteristic features of many intracellular parasite infections is their chronicity indicating that the host immune system is not capable of eradicating the pathogen. *Toxoplasma gondii* is the most successful parasite worldwide, infecting an extraordinarily broad range of hosts (endothermic animals and humans) and almost all cell types. Recent studies have revealed that in late chronic toxoplasmosis CD8+ T lymphocytes become progressively exhausted and this dysfunction is suggested to be responsible for the reactivation of latent infection, which may result in a life-threatening disease in immunocompromised individuals (e.g. neurotoxoplasmosis in AIDS patients). The article presents selected aspects of a new paradigm – T cell exhaustion phenomenon – a progressive dysfunction over time, which makes the host unable to control intracellular pathogen infections or tumours.

Key words: *T. gondii*, chronic toxoplasmosis, T lymphocyte exhaustion

*Toxoplasma gondii* and acquired host immune responses to the parasite

*Toxoplasma gondii* is recognized as the most successful parasite on the Earth. This protozoan (phylum Apicomplexa), an obligate intracellular parasite, is able to infect any nucleated cell of many endothermic animals including humans. Birds and mammals serve as intermediate hosts whereas Felidae are definitive hosts. Unlike other Apicomplexa, the parasite could be transmitted exclusively from one intermediate host to another and uses sophisticated mechanisms to keep a balance between the stimulation of host immune response and evasion. *T. gondii* is of great medical and veterinary importance and poses a severe risk for fetuses infected *in utero* and immunocompromised individuals as well as for farm animals, causing considerable economic losses due to abortions. In most immunocompetent hosts primary *T. gondii* infection occurs asymptomatically but at the end of the acute infection stage, fast replicating tachyzoites, under the pressure of generated specific cellular immunity, convert into slow-replicating, “dormant” forms, bradyzoites which enclosed in tissue cysts colonize preferentially brain, muscles and eyes [1].

The immune responses in *T. gondii* infection, local and systemic, are strongly dependent not only on the parasite genotype but also on the genetic background and the immune status of the infected host. *T. gondii*-specific T cells become activated and acquire their effector functions, including the production of cytokines such as IFN-γ and TNF-α, as well as cytotoxicity mediated by granzyme(s) and perforin. After a vast majority of the effector T cell population dies by apoptosis, the surviving T cells differentiate into a pool of long-lived memory T cells, which should retain the ability to rapidly activate effector functions and expand robustly upon the re-exposure to parasite antigen [2,3]. Both CD4+ and CD8+ T cells are crucial for host

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protection during *T. gondii* infection, however, the finding that depletion of CD8+, but not CD4+, T cells during chronic infection led to increased mortality confirmed the especial importance of these lymphocytes in long-term resistance to toxoplasmic encephalitis. CD8+ T lymphocytes were the dominant effector cells for control of *T. gondii* and produced pivotal protective cytokine, IFN-γ [4]. The results on the role of CD8+ T cells in infections caused by other protozoan parasites (*Plasmodium*, *Leishmania*) are ambiguous [5].

Suppression of the host immune response by *Toxoplasma gondii* has been observed in both human and experimental murine toxoplasmosis. Major soluble factors responsible for the immune down-regulation in *T. gondii*-infected mice are two anti-inflammatory cytokines i.e. IL-10 and TGF-β, which impair macrophages activation and enable intracellular survival of the parasite [6,7]. Effective CD8+ response is critical for the control of both acute and chronic toxoplasmosis but, as observed in C57BL/6 inbred mice (innately susceptible to *T. gondii* infection), the CD8+ response proved ineffective in protecting animals infected with low-virulent *T. gondii* ME49 strain [8]. The mice succumbed about 7 weeks after infection exhibiting a high expression of tachyzoite-specific gene (e.g. SAG1), which is a sensitive marker of parasite bradyzoite-tachyzoite interconversion followed by reactivation of infection. Why do CD8+ cells fail to fulfil their protective role in late chronic toxoplasmosis?

### T cell exhaustion in toxoplasmosis

As recently found, during late chronic toxoplasmosis CD8+ T cells become functionally exhausted. They gradually lose polyfunctionality i.e. capability of simultaneous displaying three activities: cytotoxicity (granzyme B) and production of proinflammatory cytokines (IFN-γ and TNF-α) and two proinflammatory cytokines (IFN-γ and TNF-α) production and then they transform from trifunctional cells into bifunctional, monofunctional and even non-functional ones. The frequency of polyfunctional cells declines sharply in both spleen and brain (Table 1). Besides, CD8+ T cells exhibit increased apoptosis, poor recall antigen response accompanied by an increased expression of the inhibitory receptor PD1 (Programmed Dead protein 1; PD279) and decreased expression of two transcription factors: T-bet and Eomesodermin (Eomes), both with a domain binding DNA, T-box [8,9]. PD-1 is a cell surface receptor of the immunoglobulin superfamily found on immune effector cells. Binding of PD-1 to one of its ligands, PD-L1 or PD-L2, on antigen-presenting cells, is known to negatively regulate T-cell receptor signaling and inhibit T-cell activation. Prolonged treatment with anti-PD-L1 antibody prevented the mortality of C57BL/6 mice, innately susceptible to toxoplasmosis. CD8+ expressing an intermediate level of PD-1 receptors (PD-1int) were highly responsive to the treatment and were rescued, while for PD-1high cells the therapy was less efficacious [8,9,10,11].

Interestingly, the costimulatory pathway CD40-CD40L plays a critical role in the rescue of exhausted cells under PD1 antibodies action [12]. Adoptive transfer of functional CD8+ cells from acutely infected donors to chronically infected hosts blocked the de-encystation process only transiently but it was insufficient to rescue of exhausted endogenous CD8+ cells. After the deletion of donor

<table>
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<th>Characteristics</th>
<th>Acute/early chronic</th>
<th>Late chronic/reactivated</th>
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<tr>
<td>CD8+ lymphocytes</td>
<td>Function (naive → activated)</td>
<td>Exhausted</td>
</tr>
<tr>
<td>Polyfunctionality degree#</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td><em>T. gondii</em> antigens load</td>
<td>High → Low</td>
<td>High</td>
</tr>
<tr>
<td>Recall <em>T. gondii</em> antigens reaction</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Inhibitory receptors (e.g. PD1)</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Transcription factors (T-bet, Eomes)</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Low</td>
<td>High</td>
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# CD8+ lymphocytes simultaneously displaying three activities: cytotoxicity (granzyme B) and production of proinflammatory cytokines (IFN-γ and TNF-α)
cells, a de-encystation and host mortality occurred again [13]. It is still not clear if the exhaustion in toxoplasmosis is genetically determined or it is induced only by specific microenvironmental factors (e.g. inflammatory agents, persistent presence of T. gondii antigens). Because CD4+ T cells fulfill accessory function in the maintenance of highly expressed CD8+ mediated immunity, an important issue which needs to be explained is the role of CD4+ lymphocytes in the regulation of CD8+ cells exhaustion.

T cell exhaustion is a common immune phenomenon

Influencing the course of T. gondii and other apicomplexans infections: immunosuppression initiated as soon as in the early acute stage as well as T cell exhaustion observed in the late chronic stage are two immune phenomena associated with toxoplasmosis. The first phenomenon was observed in our experiments performed on two murine inbred strains, which differ in their innate susceptibility to toxoplasmosis [14]. In the acute phase of toxoplasmosis splenocytes were found to be totally inactive – they responded neither to the natural polyvalent T. gondii antigen (Toxoplasma lysate antigen, TLA) nor T cell mitogen (phyto-hemagglutinin, PHA). The immune suppression state lasted longer in C57BL/10 mice (naturally susceptible to toxoplasmosis) than in BALB/c mice (innately resistant to toxoplasmosis). The biological role of immune suppression seems clear: first – enabling the parasite colonization of the new host and secondly – avoiding an overstimulation of the host immune system by parasite antigens and preventing excessive and pathological inflammatory response. On an experimental Alzheimer disease (AD) model it has been recently found that latent chronic T. gondii infection in the mouse brain leads to an increase in the anti-inflammatory cytokine level (IL-10 and TGF-β), decreases β-amyloid deposition and suppresses the pathogenesis of AD [15]. In general, whereas in chronic viral infections the persistent high virus level is observed, T. gondii infection is characterized by a short-term parasitemia in the acute stage, which is restricted in early chronic toxoplasmosis but returns during reactivation of chronic infection leading to dissemination of the parasite throughout the host body and resulting in high mortality. T-cell exhaustion, first described in chronic viral infection including HIV and hepatitis C, was then reported in protozoan parasite infections [reviewed in 16] and in solid as well as hematologic cancers, e.g. chronic lymphocytic leukemia, CLL [17,18]. T lymphocytes from CLL patients, of both CD4+ and CD8+ phenotype, revealed increased expression of exhaustion markers (PD-1, CD244 and CD160), and CD8+ cells were impaired in their proliferation and cytotoxic capacity. The latter resulted from granzyme packing defect and non-polarized degranulation of CD8+ effector cells. Surprisingly, in contrast to viral and parasitic infections, CLL CD8+ lymphocytes showed normal IL-2 production, an increased level of IFN-γ and TNF-α production and an elevated expression of T-bet transcription factor. Summarizing, these CD8+ cells do not exhibit all typical exhaustion features and thus could be named “pseudoehausted”. Exhausted, in contrast to effector and memory, CD8+ T cells in chronic virus infection do not up-regulate CD25 expression following IL-12, IL-18, and/or IL-21 exposure; they lose their susceptibility to antigen-independent activation by cytokines, which compromises their ability to detect bacterial co-infections [19]. Although up-regulation of inhibitory receptors is suggested as a major intrinsic factor of T cell exhaustion, additionally some extrinsic factors should be included: immunosuppressive cytokines, altered antigen presentation (by non-professional antigen-presenting cells or tolerogenic dendritic cells) or suppression of T cell immunity by regulatory T cells (Tregs) [20].

In the last years the symptoms of T-cell exhaustion in viral, bacterial, parasitic and tumour diseases were recognized but up to now detailed mechanisms underlying the phenomenon still remain unknown. The common feature of T cell exhaustion is the occurrence in antigen persistence conditions (continuous antigenic stimulation) but exhausted T cells maintain the phenotype acquired during chronic infection and transmit it to daughter cells even after antigen withdrawal [21]. Undoubtedly, new therapeutic agents effectively inducing and maintaining protective long-lasting CD8+ response of the host need to be developed to combat T. gondii and other intracellular pathogens infections as well as tumours.

References


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