

Effects of *Toxoplasma gondii* Infection on the Brain

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***Toxoplasma gondii*, an intracellular protozoan parasite, can infect humans in 3 different ways: ingestion of tissue cysts, ingestion of oocysts, or congenital infection with tachyzoites. After proliferation of tachyzoites in various organs during the acute stage, the parasite forms cysts preferentially in the brain and establishes a chronic infection, which is a balance between host immunity and the parasite's evasion of the immune response. A variety of brain cells, including astrocytes and neurons, can be infected. In vitro studies using non-brain cells have demonstrated profound effects of the infection on gene expression of host cells, including molecules that promote the immune response and those involved in signal transduction pathways, suggesting that similar effects could occur in infected brain cells. Interferon- γ is the essential mediator of the immune response to control *T. gondii* in the brain and to maintain the latency of chronic infection. Infection also induces the production of a variety of cytokines by microglia, astrocytes, and neurons, which promote or suppress inflammatory responses. The strain (genotype) of *T. gondii*, genetic factors of the host, and probably the route of infection and the stage (tachyzoite, cyst, or oocyst) of the parasite initiating infection all contribute to the establishment of a balance between the host and the parasite and affect the outcome of the infection.**

Key words: toxoplasmosis/toxoplasmic encephalitis/cyst/cell-mediated immunity/genotype/major histocompatibility complex

Introduction

Toxoplasma gondii is an extremely widespread, and thus successful, protozoan with a complex lifecycle involving felines, in which sexual development occurs, as its definitive host. Humans become infected in one of 3 ways: by ingesting *T. gondii* tissue cysts (containing bradyzoites) present in the undercooked meat (especially lamb and pork) of infected food animals; by ingesting highly infectious oocysts (containing sporozoites) present in water, garden soil, children's sandboxes, etc, contaminated by infected cat feces; or through congenital transplacental transmission of rapidly replicating tachyzoites from mothers who become infected during pregnancy (eg, by changing the cat litter) and pass the infection to the fetus.

A possible outcome of congenital infections is severe neurological and ophthalmological disease. The outcome of the other 2 modes of infection is usually a chronic, latent infection that persists for life. This latent infection has been assumed, until recently, to be clinically asymptomatic; as indicated in the accompanying articles, this assumption is being reconsidered.

By definition, latent infections involve a complex interplay between parasite and host, producing some degree of harmony. In humans, *T. gondii* performs a delicate balancing act that involves, on the one hand, modification of its proximal (and perhaps distal) environment in ways to promote its survival and transmission and, on the other hand, avoidance of overt tissue damage (directly from the parasite or indirectly from the immune response) that would lead to the demise of its host. In the vast majority of *T. gondii* infections, the parasite: host homeostasis is effectively achieved, resulting in a latent, subclinical infection. A variety of parasite and host factors can influence this balance, however, resulting in effects that can range from subtle to profound. In this review, we discuss the parasite and host determinants that influence the outcome of infection and the effects of these determinants on the brain.

Effects on the Brain

Once it enters the body, *T. gondii* traverses the intestinal or placental epithelium as a free parasite by paracellular transmigration¹ and enters circulating cells such as

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macrophages^{2,3} or dendritic cells.^{3,4} It then appears to use such cells as a “Trojan horse” to gain access to privileged sites such as the brain.

In vitro studies using mouse brain cells have demonstrated that tachyzoites invade microglia,^{5,6} astrocytes,^{7,8} and neurons,^{6,9} and the parasite thereafter forms cysts within these cells.^{6,8} An in vitro study using human neurons and astrocytes showed that *T. gondii* also forms cysts in these cells.¹⁰ Human cell division autoantigen-1 was recently identified as a key host determinant of bradyzoite development within human fibroblasts.¹¹ Electron microscopy studies on brains of chronically infected mice demonstrated that the majority of cysts are in neurons^{12,13}; the cysts were identified within axons, dendrites, or the cell body of the neurons.¹³ In mice with congenital toxoplasmosis, cysts were also found within neurons in their brains.¹⁴ In humans, proliferating tachyzoites have been detected in glial cells in a patient who had developed toxoplasmic encephalitis.¹⁵ In another case of toxoplasmic encephalitis, *T. gondii* bradyzoites were observed in a Purkinje cell in the cerebellum.¹⁶ *Toxoplasma gondii* cysts have also been reported in astrocytes in humans¹⁷; in that study, astrocytes were the only cell type that could be identified due to the poor preservation of the samples. Collectively, these studies demonstrate that *T. gondii* can infect a variety of brain cells, but additional studies are needed to identify the host cells that preferentially harbor cysts within the brain.

The effects of *T. gondii* on brain cells can be almost immediate, as shown by the work of Blader et al,¹⁸ who used tachyzoites of a type II strain to examine host gene expression profiles in infected human fibroblasts. Within the first 2 hours of infection, although <1% of the 22 000 known human genes examined were upregulated by >2-fold, almost half of the affected genes encoded proteins associated with the immune response. Included among the upregulated genes were those encoding chemokines (GRO1, GRO2, LIF, and MCP1) designed to recruit immune cells, cytokines (IL-1 β and IL-6) capable of activating immune responses, and transcription factors (REL-B, NF- κ Bp105, and I- κ B α) that can promote expression of additional immune regulators. Thus, it is clear that the host cell mounts a strong response directed at alerting and activating the immune system to react to the infection.

Twenty-four hours postinfection, by which time the parasite has replicated 2–4 times, a variety of host glycolytic and mevalonate metabolic transcripts are upregulated, presumably, in response to the nutritional drain imparted by the infection. Intracellular tachyzoites are also known to manipulate a variety of signal transduction pathways related to apoptosis,^{19–21} antimicrobial effector mechanisms,^{22–25} and immune cell maturation.²⁶ The recent finding of delivery of protein phosphatase 2C released from rhoptries of tachyzoites into the host nucleus²⁷ will likely be a key step forward toward under-

standing the molecular basis of such transcriptional manipulation. Although similar studies on brain cells have not been reported, it seems likely that *T. gondii* infection may also influence signaling pathways in the brain.

There is only limited information on manipulation of host cells by bradyzoites. Foudts and Boothroyd²⁸ recently reported that many of the same host genes (eg, cytokines and chemokines) are affected by infection with bradyzoites or tachyzoites in human fibroblasts; however, the number of genes and the magnitude of activation were both lower in bradyzoite infection. Future gene expression studies on tachyzoite and bradyzoite infection of brain cells may reveal cell type-specific changes influencing the secretion of not only cytokines and chemokines but also neurotransmitters, receptors, ion channels, and other central components of brain physiology.

Elevated anti-*T. gondii* IgG antibody levels have been reported in patients with first-onset schizophrenia,^{29,30} suggesting an involvement of this parasite in the etiology of schizophrenia. Elevated serum levels of IL-1 β have also been detected in individuals with acute schizophrenia, but not chronic schizophrenia,³¹ and there were no differences in IL-1 β or IL-6 serum or cerebro-spinal fluid levels in medicated patients compared with a control group.³² Because tachyzoites induce more pronounced inflammatory cytokine responses in host cells than do bradyzoites, as described above, proliferation of tachyzoites in the brain may be related to the onset of schizophrenia. The lack of elevated IL-1 β or IL-6 in medicated patients could be due to the antitoxoplasmic activity of some antipsychotic drugs.^{33,34} Interestingly, anti-*T. gondii* IgM antibody, a key indicator of acute acquired infection, is not elevated in the sera of patients with first-onset schizophrenia,^{29,30} implying that the patients are not in the acute stage of a newly acquired infection. Therefore, a reactivation of chronic infection with the parasite (proliferation of tachyzoites caused by cyst rupture) in the brain might be involved in the onset of the disease. In support of this possibility, expression levels of proinflammatory cytokines, including IL-1 β and IL-6, are higher in the brains of a mouse strain in which tachyzoite proliferation occurs in this organ during the later stage of infection compared with the brains of another mouse strain that prevents tachyzoite proliferation during chronic infection.³⁵ It is noteworthy that individuals with congenital *T. gondii* infection often develop ocular toxoplasmosis later in life,³⁶ and the disease is considered to be due to reactivation of infection. The onset of toxoplasmic chorioretinitis is most frequent during the ages of 20–30,³⁶ correlating well with the age of onset of schizophrenia.³⁷ Therefore, congenital infection with *T. gondii* may be involved in the etiology of schizophrenia.

Determinants of the Outcome of Infection

A variety of parasite and host factors determine the outcome of infection. When these factors are in balance,

a chronic latent infection results. When they are out of balance, active disease may ensue. The most important factors appear to be the mode of infection, parasite strain, host cytokine response, and host genes.

Mode of Infection

It is known that bradyzoites, sporozoites, and tachyzoites show pronounced differences in gene expression, cell invasiveness, replication rate, and migratory proficiency. It thus seems likely that the course of infection and clinical manifestations may be strongly influenced by the mode of the initial infection. Because congenital infections with tachyzoites produce a distinct clinical picture, including chorioretinitis and neurologic disturbance, which can be discovered later in life even when the infection is asymptomatic at birth,³⁶ it is also possible that ingesting tissue cysts containing bradyzoites or ingesting oocysts containing sporozoites may produce different clinical outcomes. The outcomes may also be influenced by the timing of the infection, such as before or after birth.

Parasite Strain

Strains of *T. gondii* have been classified into 3 major genotypes (types I, II, and III) based on polymorphisms of their genes.³⁸ Mice infected with type II strains develop toxoplasmic encephalitis after immunosuppressive treatment with anti-interferon- γ (IFN- γ) antibody, whereas animals infected with a type III strain do not.³⁹ Type II is the predominant strain isolated from patients with AIDS, from non-AIDS immunocompromised patients with toxoplasmic encephalitis, and from those with congenital infections.^{40–42} By contrast, isolates from outbreaks of acute toxoplasmosis, which show a tendency to cause severe ocular disease, are frequently type I.⁴³ Thus, the parasite genotype appears to be an important factor influencing the outcome of clinical illness in humans. If congenital infection with *T. gondii* is involved in the etiology of schizophrenia, as discussed above, this would implicate type II strains in the etiology of schizophrenia. Because type I strains have a general tendency to grow more aggressively than type II and III strains in host cells, including human fibroblasts in vitro, the aggressiveness of type I tachyzoites might also contribute to the development of schizophrenia. Studies using murine models have demonstrated that the strain (genotype) of the parasite affects the immune responses of infected cells and hosts, the IL-12 response by macrophages following infection in vitro,⁴⁴ the recognition of infected cells by T cells in vitro,⁴⁵ and the cytokine response of spleen cells and within the brains of infected mice.^{46,47}

Host Cytokine Response

Among the cytokines produced in response to *T. gondii* infection, IFN- γ is the most important. The proliferation of tachyzoites during the acute stage of infection is sup-

pressed by IFN- γ -dependent, cell-mediated immune responses^{48–50} and, to a lesser degree, by humoral immunity.^{51–53} This leads to the development of chronic infection characterized by *T. gondii* cysts, primarily in the brain. The immune responses are essential for maintaining the latency of chronic infection. Individuals with immunodeficiencies such as AIDS are at risk for reactivation of infection and the development of life-threatening toxoplasmic encephalitis.^{54–55} Murine models of the disease have demonstrated that IFN- γ is essential for the prevention of reactivation and development of toxoplasmic encephalitis.^{56–58} Cyst rupture has also been observed in chronically infected immunocompetent mice, although it is extremely rare.⁵⁹ The incidence of cyst rupture in the brain may be higher in mice congenitally infected with the parasite.⁶⁰ In these cases, however, the immune response probably limits proliferation of the parasite.

The main source of IFN- γ are T cells, which infiltrate into the brain following infection.^{61–65} IFN- γ production by this lymphocyte population is essential for preventing the reactivation of infection.^{64,65} T cells bearing T-cell receptor V β 8 are the most numerous population that produces IFN- γ in the brains of infected mice that are genetically resistant to development of toxoplasmic encephalitis.⁶⁶ Furthermore, adoptive transfer of V β 8⁺ T cells alone into infected nude mice (which lack T cells) prevents the development of toxoplasmic encephalitis.^{66,67} Thus, in murine models, the parasite antigens recognized by this T-cell population appears to play a crucial role in the induction of the protective T-cell responses to prevent reactivation of infection. In addition to T cells, other cells also must produce IFN- γ to prevent reactivation of chronic infection.⁶⁸ Microglia and blood-derived macrophages are the major non-T-cell populations that produce this cytokine in the brain of infected mice.⁶⁹

Both human⁷⁰ and murine microglia⁵ inhibit intracellular replication of tachyzoites in vitro when activated by IFN- γ plus lipopolysaccharide. Nitric oxide (NO) production by inducible NO synthase is important for the inhibitory effect of activated murine microglia.⁵ In contrast, NO is not involved in the inhibitory effect of human microglia,⁷⁰ and the mechanisms of their inhibitory effect are not yet known.

Human astrocytes activated by IFN- γ plus IL-1 β inhibit tachyzoite replication in vitro through their production of NO.⁷¹ In addition, IFN- γ and TNF- α synergistically induce an expression of indoleamine 2,3-dioxygenase (IDO) in human glioblastoma cell lines and naïve astrocytes, and this IDO activity results in strong toxoplasmostatic effects through the depletion of intracellular pools of tryptophan.⁷² IFN- γ -activated murine astrocytes also prevent the intracellular multiplication of tachyzoites; their inhibitory effect is not mediated by NO or IDO but by IGTP, a IFN- γ -inducible

GTPase of the p47 family.⁷³ More recently, Martens et al⁷⁴ showed that several p47 GTPases are recruited to the parasite-containing vacuole, where they coordinate membrane vesiculation and destruction of the parasite in murine astrocytes.

In addition to IFN- γ , infection with *T. gondii* induces a variety of other cytokines by microglia,^{75–78} astrocytes,^{75,77} and neurons.^{75,79} These may promote (eg, IL-1 and TNF- α) or suppress (eg, IL-10 and TGF- β) the inflammatory response. These cytokines appear to play an important role in regulating the resistance of hosts against *T. gondii* infection in the brain. Although T cells are the predominant lymphocyte population in the brains of infected animals, B cells,⁸⁰ NK cells,^{63,69} macrophages,^{69,80,81} and dendritic cells^{3,69,82,83} also infiltrate into the brain after infection.

Host Genes

Susceptibility and resistance to chronic *T. gondii* infection in the brain is under genetic control in both mice and humans. In mice, the *L^d* gene in the D region of the major histocompatibility complex (H-2) is important for resistance to development of toxoplasmic encephalitis.^{84,85} Resistance of mice to the disease is associated with the formation of fewer *T. gondii* cysts in the brain.^{84–86} In humans, HLA-DQ3 appears to be a genetic marker of susceptibility to development of cerebral toxoplasmosis in AIDS patients⁸⁷ and congenitally infected infants,⁸⁸ whereas DQ1 appears to be a genetic marker of resistance.⁸⁷ Because the *L^d* gene in mice and the *HLA-DQ* genes in humans are part of the major histocompatibility complex that regulates the immune responses, the regulation of the immune responses by these genes appears to be important to determine the resistance/susceptibility of the hosts to the development of toxoplasmic encephalitis.

Conclusions and Future Research

The outcome of *T. gondii* infection is strongly influenced by both parasite and host determinants. Parasite strains can differ greatly in their aggressiveness during infection and their propensity to form cysts for long-term survival. With respect to the parasite's ability to influence host gene expression, it is likely that some of these effects are universal, whereas others are cell-type specific. Future research should extend such studies to various types of brain cells and examine differences between bradyzoite and tachyzoite effects on host gene expression. For controlling *T. gondii* infections, the host critically relies on IFN- γ produced by multiple populations of immune cells, which helps infected cells limit growth of the parasite. Genetic studies also suggest that regulation of the immune response by the major histocompatibility complex probably plays an important role in the susceptibility/resistance to disease. Given the strong influence of

both parasite and host on the outcome of infection, it remains to be seen whether specific combinations of parasite and host determinants can uniquely affect brain physiology, as well as psychiatric disorders.

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