Trends in Parasitology

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Opinion

Lifelong Persistence of *Toxoplasma* Cysts: A Questionable Dogma?

Solène Rougier,[1] Jose G. Montoya,[2,3] and François Peyron[1,*]

It is believed that infection by *Toxoplasma gondii* triggers a lifelong protective immunity due to the persistence of parasitic cysts which induce immunoprotection against reinfection. A review of the scientific literature since the 1950s did not yield any definitive data regarding the duration of cysts in the host or the presence of lifelong protective immunity, which led us to question this dogma. We put forward the hypothesis that sustained immunity to *T. gondii* requires repeated antigenic stimulations. The decline of seroprevalence recently observed in many countries might contribute to explain the loss of immunity. We address the potential consequences of this phenomenon, should it persist and worsen.

Trends

Infection with *Toxoplasma gondii* is believed to induce lifelong protective immunity through long-term persistence and regular rupture of *T. gondii* cysts.

Lifelong persistence of cysts and immunity remains to be demonstrated.

We propose that sustained immunity to *T. gondii* requires repeated antigenic stimulations.

Once Infected, Forever Protected?

T. gondii is an obligate intracellular protozoan that infects about a third of the world's population, making it one of the most successful parasites in humans. Infection is generally benign, except in the foetus – resulting in severe ocular and neurological sequelae – or in immunocompromised patients, resulting in encephalitis [1]. *T. gondii* has three infective stages: (i) the oocyst, excreted in the faeces of felids, the definitive hosts, and the source of environmental contamination; (ii) the tachyzoite, the pathogenic and rapidly replicating form in nucleated cells, responsible for symptoms during primary infection or reactivation; and (iii) the bradyzoite, produced by the conversion of tachyzoites, enclosed within tissue cysts, distributed throughout the body [2]. These cysts are believed to persist for the lifetime of the host and induce a protective but not sterilizing immunity [3]. The surrogate marker for protection is the detection of *Toxoplasma*-specific antibodies. Therefore, a pregnant woman with negative serology is considered to be at risk of being infected. She should be informed about primary prevention of infection and, in some settings, regularly tested for *T. gondii* antibodies [2].

In case of positive serology before pregnancy, the commonly admitted rule is to consider the woman protected. This dogma, which has been popularized as 'once infected, forever protected', has never been questioned in the 40–50 years of its existence. However, some rare cases of congenital infection have been reported in the offspring of immunocompetent mothers who displayed positive serology before pregnancy, indicating immunity to *T. gondii*, and in one case an atypical strain was identified as infecting the newborn [4]. Recently, multilocus genotyping demonstrated an important genetic diversity among parasitic isolates, raising the question of absence of cross-protection between genetically different isolates [5], and, in fact, South American strains are able to kill or reinfect vaccinated or chronically infected mice [6].

In 1992, France implemented mass screening for the detection of toxoplasmosis in pregnant women, and since then all cases of maternal infection and congenital toxoplasmosis are detected and followed up [7]. Over the last few years, among a cohort of 700 patients with

[1]Hospices Civils de Lyon, Institut de Parasitologie et Mycologie Médicale, Hôpital de la Croix Rousse, F-69317 Lyon, France
[2]Department of Medicine and Division of Infectious Diseases, Stanford University School of Medicine, Stanford, CA 94305, USA
[3]Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory, Palo Alto, CA 94301, USA

*Correspondence: francois.peyron@chu-lyon.fr (F. Peyron).

congenital toxoplasmosis followed in our facility, we observed a decrease and negativation of IgG titers, as well as negativation of cellular immune response in congenitally infected persons (S. Rougier master thesis, Université Claude Bernard Lyon 1, 2015) (Box 1). This prompted us to search the literature for data that indicated the lifetime persistence of cysts and immunity and how this was demonstrated. Our search did not yield any definitive data regarding duration of cysts in the host or the presence of lifelong protective immunity. This raised an important point: are congenitally infected persons protected for their lifetime and, more specifically, are congenitally infected women who are considering pregnancy protected if they have no specific IgG or specific cellular response? In this opinion paper, we propose a hypothesis to explain the decrease in immune response we observed, and discuss its implications to *Toxoplasma* counseling of pregnant women.

T. gondii Life Cycle and the Immune Response

The life cycle of *T. gondii* (Figure 1) occurs in cats and other felids, where sexual replication occurs, and warm blooded animals, including humans and birds, where asexual replication can take place. Humans are infected by the ingestion of either oocysts or cysts. After ingestion, the parasite emerges from oocytes or tissue cysts and invades the intestinal mucosa, where it becomes a rapidly replicating tachyzoite and initiates mucosal immunity [3,8]. The presence of intracellular tachyzoites in the intestinal mucosa stimulates the recruitment of inflammatory monocytes, macrophages, neutrophils, and dendritic cells (DCs) [9–12]. DCs are believed to be an important mediator of parasite dissemination throughout the different tissues of the host organism. *T. gondii* tachyzoites can invade almost any nucleated cell by an active process. In infected cells, the parasites are contained in a parasitophorous vacuole where they divide by endodyogeny. After several replications, tachyzoites can either exit the cell or differentiate into bradyzoites. This transformation occurs under pressure from the immune system and possibly other unknown stress factors. Bradyzoites replicate slowly within cells mainly in the brain, muscles, and retina and form tissue cysts, which are enclosed by a thick wall and that are thought to last throughout the lifespan of the infected host. Though bradyzoites have a latent metabolism, cysts are not static stages as they can rupture and release parasites [13–16]. Although the reasons for cyst rupture and the nature of the released antigens are poorly understood, this phenomenon is considered to induce a continuous stimulation of the immune system in immunocompetent hosts, which leads to a protective, albeit not sterilizing, immune response [5].

Box 1. Case Studies

Two patients with congenital toxoplasmosis were regularly followed up in our outpatient department. At each visit, clinical evaluation, funduscopy (a test that allows examination of the fundus of the eye) and serological tests were performed.

Case study 1 (Figure IA): a woman born in 1983 presented with congenital toxoplasmosis due to late-pregnancy seroconversion with no antenatal treatment. At birth, fundus examination and cranial ultrasonography revealed no abnormalities. She was given pyrimethamine, sulphonamides, and folinic acid for 1 year. In 2002, a test for specific cellular immunity using flow cytometry to detect CD25-expressing leukocytes was positive for this patient, indicating an effective cellular response against *Toxoplasma* [61]. In 2014, 7 months pregnant, she was found with positive serology, but with a negative cellular immunity test. In July 2014, she gave birth to a healthy baby. Her serology and cellular immunity test were negative. In December 2014, the humoral response remained negative, as well as interferon-γ secretion after 48 and 72 h of lymphocyte stimulation. Plans for a second pregnancy are currently under way and the patient was advised to consider herself as nonprotected against toxoplasmosis.

Case study 2 (Figure IB): A woman born in 1989 with congenital toxoplasmosis due to maternal infection in the 18th week of pregnancy. The mother was treated with spiramycine and the patient was given pyrimethamine, sulphonamides, and folinic acid for 1 year. Funduscopy and cranial ultrasonography were normal at birth. She experienced two uneventful pregnancies in 2013 and 2015. After her second pregnancy, humoral and cellular tests were negative.

Decline in antibody titers does not necessarily mean loss of protection, as it can be due to a lack of sensitivity of the test. The negativation of the cellular response could have been explained by the shifts in balance in type 1/type 2 immune responses observed during pregnancy [62–65]. In both cases the cellular response remained negative after delivery. When combined with a negative serological test, a negative cellular response raises the question of persistence of protection over time, which is a real concern in women of child-bearing age.