Pathophysiology of Toxoplasmosis

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Toxoplasma infection in most adult animals and humans is asymptomatic because of effective protective immunity; this involves antibody acting extracellularly, and T-cell factors acting intracellularly. Whenever immunity is not acquired in a timely fashion, tachyzoites continue to multiply, destroying an excessive number of cells, producing lesions in several organs, with pneumonia and encephalitis the prominent causes of illness and death. However, immunity is insufficient to destroy the slowly multiplying bradyzoites persisting in tissue cysts in many organs— a parasite adaptation to await ingestion of one host by another.

Toxoplasma cysts produce lesions when they disintegrate, because of the delayed type of hypersensitivity accompanying infections. In the presence of immunity, the released bradyzoites are destroyed, but when protective immunity fails, the bradyzoites can develop again into actively multiplying tachyzoites parasitizing and destroying cells in expanding foci, usually in the brain. In this review J.K. Frenkel discusses the complex interplay of immunological and parasite factors participating in the various lesions associated with acute and chronic Toxoplasma infections.

Toxoplasma gondii is an unusual organism that has probably evolved from a classical coccidian of cats to parasitize a wide variety of mammal and bird cells. Like other coccidia, it undergoes a proliferative and a sexual cycle in the intestine, which result in the formation of egg-like oocysts shed in the faeces. However, in contrast to one-host coccidia, oocysts of Toxoplasma are not very infective to the definitive hosts, cats; instead they are regularly infective when ingested by other mammal and bird intermediate hosts, in which infection may give rise to lesions in many organs – intestine, liver, lymph nodes, lung, heart and brain. The successful cycle between cats and their prey, usually rodents and birds, depends on prolonged survival of the parasite in the intermediate host. In nature, Toxoplasma generally gives rise to asymptomatic infection; toxoplastic lesions are usually associated with a local or generalized immune defect (see below).

Pathology and pathogenesis

The pathophysiology of toxoplasmosis results from an interplay of parasite and host factors. Tachyzoites, the motile, rapidly multiplying stage, are the principal pathogenic form of Toxoplasma. They actively invade host cells by means of an anterior organelle and release of proteolytic enzymes, causing local disruption of the host cell plasmalemma. The invading organisms become surrounded by a parasitophorous vacuole within which tachyzoites divide by endodyogeny, a special type of division. By the time 8–32 intracellular tachyzoites are formed, the cell disintegrates, probably as a result of parasite competition with essential host cell processes.

Bradyzoites, or slowly multiplying zoites, are the principal form of Toxoplasma in chronic infections. Bradyzoites persist in host cells for long periods, accumulating tens to hundreds of organisms. They are enclosed in a cyst wall that develops from the parasitophorous vacuole. Bradyzoites develop from tachyzoites as early as 3–5 days in mice, in 7 days after inoculation with bradyzoites, and in 9 days after inoculation with sporozoites from oocysts. The biological importance of bradyzoites is shown by the short, 3–10 day prepatent period before oocyst shedding in cats (Figs 1 and 2); after tachyzoite or oocyst infection, the prepatent period is long, and 19–48 days may elapse before oocysts are shed.

The transformation from tachyzoites to bradyzoites consists of a gradual, noncyclic population shift, which is favoured by the development of immunity, but develops slowly and in small numbers even in cell cultures in the absence of known immune factors. Host cells can tolerate a much greater load of bradyzoites than of tachyzoites. The infected cells sometimes die, and some authors believe that cysts may persist even in the absence of host cells.

Sporozoites develop in shed oocysts in the presence of oxygen and at lower than body temperature. They remain viable...
LIFE CYCLE OF TOXOPLASMA

Fig. 1. Life cycle of Toxoplasma in cats, the definitive host, and in mice, representing intermediate hosts. Each cat represents an infection with a different inoculum. Ingestion of a mouse with Toxoplasma cysts leads to the enteric/cystic cycle in the cat intestine and persistent periods of 3-10 days depending on the infecting dose. In addition, most cats develop tissue infections indicated by tachyzoites and cysts. However, after cats ingest mice containing only tachyzoites, or sporozoites from oocysts, a generalized infection with tachyzoites develops first; later, bradyzoites are formed which initiate the enteric/cystic cycle, with a persistent period of 20-40 days to oocyst shedding. Mice represent the many intermediate hosts supporting only the tissue cycle of Toxoplasma. Tachyzoites characterize the acute infection and tissue cysts the chronic infection. Congenital transmission has been observed in man and in some animals after acute infection, and in a few species also during chronic infection.

for a year or longer in the presence of moisture. They are not pathogenic in themselves but transform into tachyzoites as soon as they enter the gut mucosa.

Cell cultures provide models of infection in the absence of acquired immunity, although cells differ in their capacity to support Toxoplasma infections. Some host cells disintegrate once parasitized by tachyzoites, whereas others support hundreds. Even the formation of cysts has been observed in culture. Infected cells may flourish and divide by mitosis.

The pathogenicity of Toxoplasma strains also plays a role in cell destruction. In general, organisms that have been passed in laboratory animals, especially as acute infections, appear more pathogenic than isolates from naturally infected hosts.

**Immunological mechanisms in toxoplasmosis**

During analysis of toxoplasmic lesions, it is important to consider why immunity did not inhibit them. Tachyzoites are the principal pathogenic stage in toxoplasmosis and an understanding of their potential immunological control is essential for an understanding of pathophysiology. Antibody lyses extracellular Toxoplasma in the presence of a complement-like accessory factor. However, transfer of even large doses of antibody generally does not protect mice against pathogenic Toxoplasma infection, nor against the pathogenetically similar Besnoitia, which is used as a model. Immunity can develop in nu-suppressed mice that do not have antibody. Activated macrophages phagocytose and kill Toxoplasma by means of oxygen-mediated mechanisms. Interferon gamma (IFN-γ) activates macrophages and confers some protection against Toxoplasma in mice, whereas monoclonal antibody against IFN-γ is immunosuppressive.

Unlike Leishmania spp which live almost exclusively in macrophages and histiocytes, Toxoplasma invades and multiplies in various tissue cells, most of which are not phagocytic. How do we explain acquired immunity in fibroblasts, hepatocytes, neurons and myocardial cells? How is specificity -- the hallmark of acquired immunity -- expressed? There are similarities of nonspecific aspects of immunity in tuberculosis, leishmaniasis and other infections that have been emphasized, but immunity against Toxoplasma is highly specific by comparison with the related but immunologically distinct protozoan Besnoitia feline spp. Hamsters are immune against 10⁶-10⁸ homologous Toxoplasma or Besnoitia parasites, but cross immunity or nonspecific immunity protects against less than ten of the parasites. A decisive role for antibody and B-cells has been excluded (see above) and the sensitivity of immunity to corticosteroids, and to as little as 50-100 roentgens prior to adoptive transfer, suggest that T-lymphocytes play the decisive role.

A comparison of lymphoid cells from hamsters immune to Toxoplasma, with those immune to Besnoitia, showed that lymphocytes expressed specificity to each but macrophages did not either in vivo or in vitro. The same specificity was found in lymphocyte supernatants. In addition, immune activity was expressed against Toxoplasma growing in fibroblasts and kidney cells -- the effective moiety appeared to be a specific mediator of about 4 kDa. The crucial role of T-cells was confirmed by finding that athymic nude mice did not develop protective im-
munity\textsuperscript{24} and that elimination of CD4 cells abolished previously acquired immunity\textsuperscript{25}.

Protective immunity is measured by its adverse effects on \textit{Toxoplasma}, whereas hypersensitivity is measured by its deleterious inflammatory effects on the host. Immunopathology plays a role in some toxoplasmic lesions. Delayed type IV hypersensitivity, the intense inflammatory reaction after re-exposure of a sensitized host, is observed in man and several experimental animals. As measured by an intradermal test, DTH can be elicited in hamsters after 3–4 days of infection, whereas protective immunity takes 2–3 weeks to develop\textsuperscript{26}. In humans the early post-infection period has not been studied, but during later infection, both protective immunity and DTH are present\textsuperscript{27}. The intense inflammatory reaction following cyst rupture has been attributed to DTH, especially in the retina\textsuperscript{27}. During later infection, even antigen release can be associated with pronounced inflammation and necrosis of uninfected cells, whereas during early infection necrosis is limited to infected cells\textsuperscript{28}.

Toxoplasmic proliferation sometimes continues in the brain and eye after extraneural tissues have become immune.

Tachyzoites in Groups
Mainly in Non-immune Host

Bradyzoites in Cysts
In Immune Host — Only?

Development of immunity.
Organisms enter new cells
Rapid endodyogeny for a few days by tachyzoites
"Groups"

Development of cyst wall intracellularly.
Cyst is non-chemotactic
Host cell degenerates.
RUPTURE OF CYST
While immunity is high.
Bradyzoites destroyed
Slow endodyogeny for weeks by bradyzoites.

In man, acquired toxoplasmosis may be totally asymptomatic, or may result in lymphadenopathy and similar "flu-like" symptoms. If the patient becomes immunocompromised, rapid dissemination may occur, leading in some cases to ocular toxoplasmosis or fatal CNS disorders.

Fig. 2. Extraintestinal or tissue cycle of \textit{Toxoplasma} and histological response. (Taken from Ref. 51.)
infection-immunity in toxoplasmosis. This cannot be generalized however, because a cyst-less strain of *B. melitensis* is not associated with sterile immunity, but with premunition.

Immunity is better expressed in the maternal mouse than in its offspring, although intra-intestinal immunization protects about 40% of foetuses. In rats and hamsters (which are more immunocompetent than mice) foetuses are better protected by a tachyzoite vaccine (J.K. Frenkel, unpublished), and this may be expected in immunocompetent humans. Oocyst shedding by cats is also under immunological control. It is found after oral infection, generally with an oocyst-producing strain, or at least some intestinal multiplication.

Host factors predisposing to lesions

*Animals that develop severe toxoplasmosis.* Although asymptomatic infection is the rule in nature with a variable percentage of cats, rats, mice, sheep and ground-feeding birds being infected, the pathophysiology of infection is best studied in the presence of symptomatic illness. Fatal infection in laboratory animals first drew attention to toxoplasmosis in 1918, when gondis (wild North African rodents) became sick and died in the Pasteur Institute of Tunis, and a laboratory rabbit in São Paulo, Brazil, died from this infection. Both probably became infected after eating food contaminated with cat faeces. Since then, fatal infection has been reported from many animal species. However, disseminated fatal infections generally occur in adults of the following groups: Australian marsupials, Madagascan lemurs, and neotropical monkeys. These animals evolved in the absence of cats or in ecological isolation from them (e.g. arboreal monkeys), whereas terrestrial neotropical animals tend to have asymptomatic infections (Ref. 37 and J.K. Frenkel, unpublished).

*Young animals.* Toxoplasmic illness is also common in young or immature animals, generally before the time the species normally comes in contact with *Toxoplasma*. Human babies infected *in utero* often become sick, whereas their mothers have asymptomatic acute infections. Foetal or young sheep and pigs often develop illness and die if infected *in utero*, whereas adults remain asymptomatic. Day-old rats and guinea-pigs have fatal infections but even young animals undergo asymptomatic infections.

*Compromised hosts.* Immunosuppressed hosts suffer infections accompanied by illness that is usually fatal. The current AIDS pandemic presents many examples of relapsing or recrudescence chronic infections. This reflects the common occurrence of asymptomatic infection in many human populations, and the AIDS-related failure to maintain earlier immunity. Other reports of relapsing toxoplasmosis come from patients immunosuppressed for transplant surgery or cancer treatment. Relapsing toxoplasmosis is usually accompanied by localized lesions, principally in the central nervous system. Primary infection in compromised hosts is a severe generalized multi-organ disease that is usually fatal, as in nude mice.

Specific aspects of pathogenesis

The normal route of infection is oral when oocysts from soil and tissue cysts from meat are ingested. The portal of entry in the gut may contain large numbers of *Toxoplasma* multiplying in the lamina propria, sometimes leading to small ulcers (Ref. 41 and F. Bertoli, unpublished). Foetal infection is parenteral via the umbilical vein from the placenta. Whether acquired orally or parenterally, infection disseminates via the lymphatics, the lymph nodes, by blood to the liver, and from there to the lung and the rest of the body.

Lesions during acute infection. Lesions from primary infection often occur in the liver. Tachyzoites enter hepatocytes and occasionally Kupffer cells, and multiply. Liver cells appear to be destroyed by 16–32 tachyzoites which can then invade adjacent cells. With time, necroses of individual cells develop into small foci of tissue necroses accompanied by mixed, but mainly mononuclear inflammatory reactions. Such focal lesions can be seen in the brain and lung of hamsters with a relapsing infection. The dead cell bodies remain and the liberated organisms invade adjacent cells. Sometimes these lesions are clearly visible, resembling a ‘bull’s eye’ or ‘target’, with dilated blood vessels or peripheral haemorrhage. The size of the lesion depends on the duration of multiplication, and the permanence of the necrotic cells, which is aided by the hypercorticotoid state. Normally, necrotic cells are lysed. Most multiplication is seen in liver, lymphoid tissues, lung and brain, suggesting good availability of substrate. In kidney and skeletal muscle, small foci of infection are found; this is not necessarily a function of substrate because in cell...
culture, kidneys and muscle cells appear to support good growth.

With the development of immunity, tachyzoite multiplication decreases and ultimately stops. A necrotic focus may be replaced by regenerating cells as in the liver, by a fibrous scar in non-regenerating tissue as in the myocardium, or by a glial scar as in the brain. Toxoplasma cysts are often seen in the neighbourhood of these scars, without causing inflammatory reaction. Apparently, antigenic material does not escape from intact cysts and the cysts are well-adapted to persist and maintain chronic infection, awaiting ingestion by a carnivore.

**Lesions during chronic infection.** When cysts that maintain chronic infection disintegrate, they often give rise to lesions with intense inflammation suggestive of delayed hypersensitivity. Although only a single host cell is destroyed by the cyst, many surrounding cells may undergo necrosis. Generally, the liberated bradyzoites are destroyed — evidence that immunity remains intact. The earliest lesions, of ‘leaking’ cysts accompanied by microglial inflammatory cells, were seen in the brain of a neotropical night monkey, Aotus lemurinus28. Disintegrating cysts, with poorly staining and partially lysed bradyzoites, were surrounded by larger glial nodules. Some nodules without stainable organisms contained Toxoplasma antigen that could be identified with antibody and visualized by the peroxidase—anti-peroxidase technique. Although cysts without any inflammation were also found, a sufficiently large number of cysts were disintegrating, so that the animal died with signs of acute encephalitis, but with intact immunity28. In hamsters chronically infected with some strains of Toxoplasma, a slowly progressive encephalopathy with chorea and a circling gait developed; after several months, the multiple superimposed spinal cord lesions resulted in posterior paralysis. This was associated with disintegration of cysts, focal necrosis and glial scars29.

Infarction necrosis is sometimes seen in the brain of human and animal patients with severe toxoplasmosis and is attributed to organisms multiplying in vessel walls, resulting in vascular thrombosis37. Periaqueductal and periventricular necrosis due to vasculitis is a unique lesion observed only in congenitally infected children with cerebral toxoplasmosis27. Following transport by blood to the brain, some Toxoplasma are shed into the ventricular system where they parasitize the ependymal cells including those of the aqueduct of Sylvius. This narrow section of the ventricular system easily becomes obstructed, leading to accumulation of ventricular fluid containing Toxoplasma antigen in the lateral and third ventricles. In the presence of ependymal ulcers, this antigen-containing fluid diffuses into the subependymal tissues, giving rise to inflammation around blood vessels. The lesions present suggest that Toxoplasma antigen in the ventricular fluid reacts with antibody within the vessels, giving rise to vascular wall oedema, leakage of plasma proteins, inflammation, thrombosis and infarction necrosis of the subependymal brain tissue27 — consistent with a type III hypersensitivity lesion. Antibody consists of IgG passively transferred from the mother, and IgG and IgM synthesized by the infant. The possibility of toxins causing the inflammation was excluded by the finding of granulation tissue extending from some vascular walls into the ventricles, actually a site of antigen excess. The lesions are most intense where both antigen and antibody can be presumed present. So far this lesion has not been reinvestigated with contemporary immunological reagents, because as most of the patients are diagnosed and treated the lesions are rarely seen at autopsy.

Periventricular necrosis was found in most babies with cerebral toxoplasmosis examined in the USA and Europe. However, I have not found this lesion in slides of about ten toxoplasmic babies from Costa Rica. These children were malnourished and it may be that antibody titres were insufficient to give rise to an antigen—antibody reaction in these babies.

Cerebral calcification is often found radiologically in babies with foetally acquired toxoplasmosis. This dystrophic calcification is superimposed on brain necrosis, both periventricular and scattered where infarction necrosis is present27.

Granulomatous inflammation with epithelioid and giant cells is often found in the choroid of enucleated eyes with chronic toxoplasmosis. This lesion formerly gave rise to mistaken diagnosis of tuberculosis or syphilis42. However, stains for tubercle bacilli and syphilis spirochaetes were negative in the choroid, and Wilder42 identified Toxoplasma tachyzoites in the retina, associated with mixed inflammatory reaction, mainly lymphocytes and monocytes. The exceptional granulomatous inflammatory reaction in the choroid can probably be explained by the sensitization to

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As pregnancy advances, so the infection rate to the foetus increases, but the severity of infection tends to decrease with advancing pregnancy. Infection risk during the third trimester of pregnancy can be over 60%, but severe foetal infections at this stage are unusual.
soluble retinal antigen and to Toxoplasma.

Immune complex nephritis has been described in some patients with congenital and ocular toxoplasmosis. Toxoplasma antigen, immune complexes and complement were found in certain patients and in the kidneys of others. Antigen–antibody complexes were found in the glomeruli of mice following primary infection but without development of progressive lesions.

Lymphoid hyperplasia, especially of cervical lymph nodes surgically removed when lymphoma is suspected, can sometimes be attributed to toxoplasmosis. Significantly, Toxoplasma is rarely seen. Instead, there is follicular and paracortical hyperplasia and proliferation of histiocytes, with histiocytes even infiltrating the hyperplastic germinal centres. The high antibody titre in these patients and the occasional history of previous illness, suggests this lesion is an example of immune hyperplasia. In humans and animals that die from toxoplasmosis, lymphoid tissues are generally depleted of lymphocytes.

Enteric toxoplasmosis in cats follows the ingestion of meat from a chronically infected animal containing cysts with bradyzoites. Minimal lesions are usually found because the length of the Toxoplasma cycle is closely attuned to the normal turnover rate of the intestinal epithelium. The principal proliferative stages enter enteroepithelial cells near the neck of the intestinal crypts and finish their multiplication before the host cells are shed at the tips of the villi. Gametocytes develop, then gametes are formed and oocysts are shed near the tips of the villi. Infection in nursing kittens is frequently fatal because of disseminated lesions or encephalitis. Once weaned, the infection is asymptomatic. However, in old cats with waning immunity, relapsing toxoplasmosis occasionally gives rise to a granulomatous mass in the muscular wall of the intestine.

Prevention of Congenital Toxoplasmosis
- Never eat undercooked or raw meat during pregnancy.
- Wash hands thoroughly after preparing meat and never put hands in your mouth.
- Wash kitchen surfaces and utensils that have contacted raw meat.
- Keep insects away from food, especially flies, as they can carry Toxoplasma.
- If you own a cat:
  - Do not feed it raw meat.
  - Get someone else to empty its litter tray if this is difficult, wear gloves. Disinfect the litter tray with boiling water for 5-10 minutes. Better still, get someone else to look after the cat during pregnancy.

References