TOXOPLASMOSIS OVERVIEW

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Content:

Dedication

To my mother & my father

Acknowledgement

To my teachers, colleagues and students.

Mosab Nouraldein

Introduction

Toxoplasma gondii, a protozoan parasite of mammals, is transmitted when oocytes excreted by cats or present in undercooked meat are ingested. Invasive forms enter the bloodstream to reach the brain, heart and lungs, where they form cystic aggregates that remain latent, but are subject to reactivation throughout the life of the host. In many communities most people have been infected by early childhood, but otherwise healthy persons do not develop clinically evident disease. In HIV-infected patients, however, toxoplasmosis holds serious implications. [(1)]

History

T. gondii was discovered by Charles Nicolle and L. Manceaux in 1908 in a North African rodent, Ctenodactylus gondii. The organism was named Toxoplasma because of its crescent shape (toxon, arc or bow as in bow and arrow, Greek, and plasma, form, Greek) and gondii after its rodent host, the gondi. At about the same time T. gondii was described from a laboratory rabbit, and then in a human in Panama. In 1909 Chatton and LeBlanc found that gondis, which live in the foothills and mountains of Tunisia, were not naturally infected, and they surmised that the infection was acquired in the laboratory. Since at the Pasteur Institute in Tunis, where Nicolle and Manceaux were carrying out research on leishmaniasis, the parasite was found in the blood, spleen and liver of the gondi, it was suspected that the rodent had acquired its infection at the Institute through the bite of an arthropod. Several investigators in Tunis and the US attempted arthropod transmission without success. Interest in human infections began in 1923 when T. gondii was described in the retina of a hydrocephalic child, and in congenital toxoplasmosis. Within 5 years Albert Sabin (of polio vaccine fame) had characterized the pathology of the congenital infection and in collaboration with Henry Feldman developed a specific serological "dye test" for toxoplasmosis. Using the dye test toxoplasmosis was determined to be a human infection worldwide in distribution.

For more than 35 years the route of infection remained a mystery since congenitally acquired infections could not explain its widespread frequency. In 1956 D. Weinman and Asa Chandler postulated that the infection was transmitted through the ingestion of undercooked meat, and shortly thereafter Leon Jacobs provided evidence for this when he showed that when cysts were exposed to proteolytic enzymes the cyst wall dissolved releasing viable infective stages. The

hypothesis was proven by the 1965 experiments of Desmonts et al.: children in a Paris TB hospital were fed either undercooked lamb chops or barely cooked beef or horsemeat. In the former group the antibody levels (by the Sabin-Feldman dye test) rose from 10-50% (which they were in the latter group) to 100%. In Paris where eating raw or undercooked meat is customary, over 80% of the population was found to be seropositive for toxoplasmosis.

There remained a perplexing question: How could one explain the similar abundance of toxoplasmosis in humans who ate meat with that of non-meat eaters i.e. vegetarians as well as herbivores? Up until 1970 the only described stages of Toxoplasma were called tachyzoites which grew in a wide variety of cells, mostly phagocytic, and which then developed into cyst like bodies, found in the brain and muscles, and containing stages called bradyzoites. When examined with the transmission electron microscope these invasive stages had an apical complex, and so clearly T. gondii was an apicomplexan. The complete life cycle was described in 1970 when the sexual stages were found in the intestinal cells of the cat and oocysts were found in cat feces. (2)

Charles Nicolle (1866-1936)

CHARLES NICOLLE

Also Known As: Dr. Charles Nicolle

Famous as: Bacteriologist

Nationality: French

Birthday: September 21, 1866

Died At Age: 69

Born in: Rouen

Died on: February 28, 1936

Place of death: Tunis (3)

Ctenodactylus gondii

The gundi is a guinea pig-sized rodent with a head-body length of 16-20 cm and a short tail of 1-2 cm. Body weight averages 175 g. The upper parts are buff-coloured, occasionally pinkish-buff, and the underparts are paler, usually whitish or slaty.

Gundis are diurnal rodents that inhabit semi-desert regions of northern Africa. They shelter in crevices within rock outcrops and live in varying sized family groups. (4)

Ctenodactylus gondii

Epidemiology

Toxoplasmosis is caused by the protozoan parasite Toxoplasma gondii. In the United States it is estimated that 11% of the population 6 years and older have been infected with Toxoplasma. In various places throughout the world, it has been shown that up to 95% of some populations have been infected with Toxoplasma. Infection is often highest in areas of the world that have hot, humid climates and lower altitudes.

Toxoplasmosis is not passed from person-to-person, except in instances of mother-to-child (congenital) transmission and blood transfusion or organ transplantation. People typically become infected by three principal routes of transmission.

Foodborne transmission:

The tissue form of the parasite (a microscopic cyst consisting of bradyzoites) can be transmitted to humans by food. People become infected by:

•Eating undercooked, contaminated meat (especially pork, lamb, and venison)

•Accidental ingestion of undercooked, contaminated meat after handling it and not washing hands thoroughly (Toxoplasma cannot be absorbed through intact skin)

•Eating food that was contaminated by knives, utensils, cutting boards, or other foods that had contact with raw, contaminated meat.

Zoonotic transmission:

Cats play an important role in the spread of toxoplasmosis. They become infected by eating infected rodents, birds, or other small animals. The parasite is then passed in the cat's feces in an oocyst form, which is microscopic.

Kittens and cats can shed millions of oocysts in their feces for as long as 3 weeks after infection. Mature cats are less likely to shed Toxoplasma if they have been previously infected. A Toxoplasma-infected cat that is shedding the parasite in its feces contaminates the litter box. If the cat is allowed outside, it can contaminate the soil or water in the environment as well.

People can accidentally swallow the oocyst form of the parasite. People can be infected by:

•Accidental ingestion of oocysts after cleaning a cat's litter box when the cat has shed Toxoplasma in its feces.

•Accidental ingestion of oocysts after touching or ingesting anything that has come into contact with a cat's feces that contain Toxoplasma.

•Accidental ingestion of oocysts in contaminated soil (e.g., not washing hands after gardening or eating unwashed fruits or vegetables from a garden).

•Drinking water contaminated with the Toxoplasma parasite.

Vertical (congenital) transmission:

A woman who is newly infected with Toxoplasma during pregnancy can pass the infection to her unborn child (congenital infection). The woman may not have symptoms, but there can be severe consequences for the unborn child, such as diseases of the nervous system and eyes.

- Blood transfusion
- Organ transplantation. [5]

Taxonomy:

Toxoplasma gondii is placed in the phylum Apicomplexa (Levine 1970), class Sporozoasida (Leuckart 1879), subclass Coccidiasina (Leukart 1879). Traditionally, all coccidia until 1970 were classified in the family Eimeriidae. After the discovery of the coccidian cycle of T.gondii in 1970, T.gondii has been placed by diff erent authorities in the families' Eimeriidae, Sarcocystidae or Toxoplasmatidae.

Life cycle:

The enteroepthelial cycle in the definitive host:

Five morphologically distinct asexual stages (types A–E) of T. gondii develop in enterocytes before Gametogony begins. The origin of the gametes has not been finally established, but it is believed that merozoites (stages D and E) develop into gametes. Gametes occur throughout the small intestine, but are most prevalent in the ileum, where they are found 3–15 days after infection.

The microgamete (the male gamete) is biflagellate and fertilizes the macrogamete (the female gamete) within the enterocyte. Oocysts are formed when a wall is laid around the fertilized gamete (zygote). Oocysts are expelled into the intestinal lumen after the rupture of enterocytes and are Unsporulated when excreted in faeces.

The prepatent period (interval between ingestion and shedding of oocysts) after the ingestion of tissue cysts is 3–10 days, with peak oocyst production between 5 and 8 days after a patent period varying from 7 to 20 days. Cats not previously infected with T. gondii shed oocysts after ingesting each of the infective stages of the parasite: the tachyzoite, the bradyzoite and the sporozoite. The prepatent period varies according to the stage of T. gondii with which the cat is infected, with a short (3–10 days) prepatent period when the oral inoculum contains bradyzoites and a long prepatent period (>13 days) when the inoculum contains tachyzoites or sporozoites. Cats previously infected with T. gondii, and which produced oocysts during the previous infection, are generally immune to renewed oocyst shedding, but immunity is not lifelong.

Fertilization initiates oocyst wall formation. The oocyst is the developed zygote, which is the product of sexual reproduction through fertilization of the macrogamete by the microgamete. The oocysts are discharged into the intestinal lumen by rupture of the epithelial cells, and thereafter excreted in cat faeces. The oocysts sporulate within 1–5 days after excretion, depending on aeration, humidity and temperature, by dividing into two sporocysts. Each sporocysts contains four sporozoites. Thus, there are eight sporozoites in one oocyst. The sporulated oocyst can remain infectious in the environment for months even in cold and dry climates.

The asexual cycle in the definitive host:

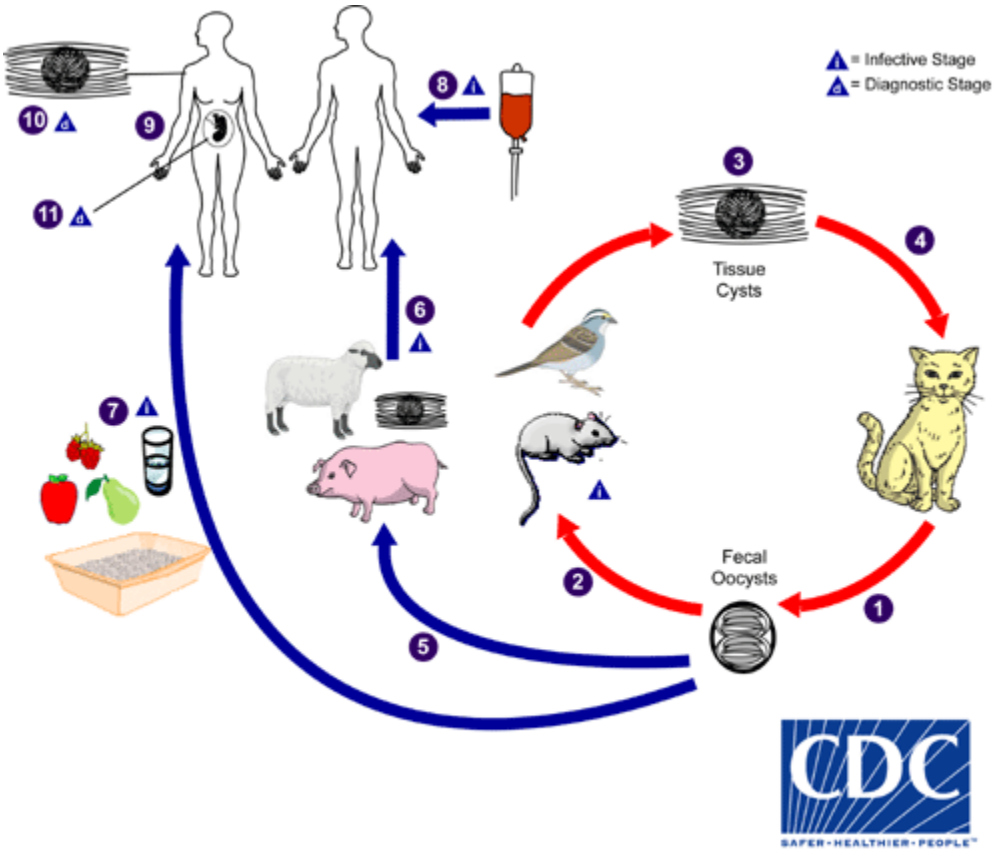
As the entero–epithelial cycle progresses, bradyzoites penetrate the lamina propria below the epithelial cell in the intestine of the cat and multiply as tachyzoites. The tachyzoites are disseminated throughout the body within a few days, eventually encysting in tissues. The extra-intestinal cycle in the cat diff ers from the similar cycle in nonfeline intermediate host in two aspects: **(1)** tachyzoites have not been demonstrated in feline intestinal epithelial cells, whereas they do occur in nonfeline intermediate hosts, and **(2)** the entero–epithelial types of T. gondii are noninfectious to mice by any route, which suggests that the feline entero–epithelial forms do not give rise to tachyzoites.

Intermediate host:

Toxoplasma gondii tachyzoites are disseminated throughout the body of the intermediate host in macrophages and lymphocytes as well as free in the plasma. Tachyzoites continue to divide within the host cell by endodyogeny (internal division into two) until the host cell is filled with parasites. At a given time the dividing tachyzoites cannot be contained within the host cell, which bursts. The tachyzoites are released and seek new host cells to repeat the process. Depending on the strain of T. gondii and the host resistance, tachyzoites may be found for days or even months after acute infection. For example, tachyzoites persist in foetal membranes for weeks after infection of the mother or the dam, and are nearly always present in placentas of mothers at the time of parturition, if the foetus was infected in utero.

At some time after infection the tachyzoites transform to bradyzoites in tissue cysts. The signals responsible for the transformation are not known, and the debate continues as to whether signals from the host immune system are needed. Bradyzoites also divide by endodyogeny. Bradyzoites are enclosed in a thin cyst wall. Tissue cysts may be found as early as 3 days after infection but are usually not numerous until 7 weeks after infection. Intact tissue cysts probably do not cause any inflammation and may persist for life. It has been suggested that tissue cysts may switch from the bradyzoite stage to the tachyzoite stage during the life of the tissue cysts, producing new tachyzoites which may give rise to new tissue cysts thus ensuring a prolonged infective stage. If the intermediate host is eaten by another warm-blooded animal, tissue cysts are able to infect a new host.

Fewer than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas almost all cats shed oocysts after ingesting tissue cysts. Cats infected with oocysts and tachyzoites probably give rise to bradyzoites, which after a variable period of time may disseminate to the intestinal mucosa and start the entero–epithelial cycle with the resulting production of oocysts. [(6)]



1-The only known definitive hosts for Toxoplasma gondii are members of family Felidae (domestic cats and their relatives). Unsporulated oocysts are shed in the cat's feces.

2-Although oocysts are usually only shed for 1-2 weeks, large numbers may be shed. Oocysts take 1-5 days to sporulate in the environment and become infective. Intermediate hosts in nature (including birds and rodents) become infected after ingesting soil, water or plant material contaminated with oocysts.

3-Oocysts transform into tachyzoites shortly after ingestion. These tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites.

4-Cats become infected after consuming intermediate hosts harboring tissue cysts.

5-Cats may also become infected directly by ingestion of sporulated oocysts. Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment.

- *Humans can become infected by any of several routes :*
  6- Eating undercooked meat of animals harboring tissue cysts
  7- Consuming food or water contaminated with cat feces or by contaminated environmental samples (such as fecal-contaminated soil or changing the litter box of a pet cat.
  8- Blood transfusion or organ transplantation.

9- Transplacentally from mother to fetus.
10- In the human host, the parasites form tissue cysts, most commonly in skeletal muscle, myocardium, brain, and eyes; these cysts may remain throughout the life of the host. Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained biopsy specimens.
11- Diagnosis of congenital infections can be achieved by detecting T. gondii DNA in amniotic fluid using molecular methods such as PCR. [7]

Pathology and pathogenesis

Pathogenesis:

Most cases of toxoplasmosis in humans are acquired by ingestion of infected meat containing tissue cysts with bradyzoites or food contaminated with cat feces containing oocysts. Bradyzoites or sporozoites penetrate intestinal cells and then spread locally to the mesenteric lymph nodes and then to distant organs via the lymphatics and blood. Focal areas of necrosis may develop in a variety of organs and the clinical manifestations reflect injury to specific tissues. Tissue death is not the result of a Toxoplasma toxin, but is a consequence of the egress of the tachyzoites which destroys the host cell. Only 10-30% of toxoplasma infections are symptomatic and the most common clinical manifestation in immunocompetent adults is lymphadenitis and lymphadenopathy.

The most common symptom is swollen lymph nodes which may be associated with fever, headache, muscle pain, anemia and sometimes lung complications. Any lymph node can be infected but commonly the deep cervical nodes of the neck are involved. There is malaise, fever, and lymphocytosis which mimics infectious mononucleosis. The infection usually resolves on its own in weeks or months. [8]

Symptoms:

Most healthy people who are infected with toxoplasmosis have no signs or symptoms and aren't aware that they're infected. Some people, however, develop signs and symptoms similar to those of the flu, including:

- Body aches
- Swollen lymph nodes
- Headache
- Fever
- Fatigue

Symptoms in immunocompromised (AIDS patients) or immunosuppressed patients (cancer, organ transplant) are more severe includes:

- Headache
- Confusion
- Poor coordination
- Seizures
- Lung problems that may resemble tuberculosis or Pneumocystis jiroveci pneumonia, a common opportunistic infection that occurs in people with AIDS
- Blurred vision caused by severe inflammation of your retina (ocular toxoplasmosis).

Babies are most at risk of contracting toxoplasmosis if their mothers become infected in the third trimester and least at risk if the mother become infected during the first

trimester. On the other hand, the earlier in pregnancy the infection occurs, the more serious the outcome for baby.

Many early infections end in stillbirth or miscarriage. Infants who survive are likely to be born with serious problems, such as:

- Seizures
- An enlarged liver and spleen
- Yellowing of the skin and whites of the eyes (jaundice)
- Severe eye infections.

Only a small number of babies who have toxoplasmosis show signs of the disease at birth. Often, infants who are infected don't develop signs — which may include hearing loss, mental disability or serious eye infections — until their teens or later. [9]

Complications

Ocular toxoplasmosis:

The toxoplasmosis infection can sometimes spread to the eyes. This is called ocular toxoplasmosis and is possible even after the initial infection. The T. gondii parasite, which causes toxoplasmosis, can lie dormant (asleep) in the retina for many years. It can wake up at any time and start a new infection.

Retinochoriditis

Ocular toxoplasmosis causes ocular lesions, which are wounds in the eyes that are caused by inflammation and scarring. These can appear in:

- The retina (the nerve tissue that lines the back of the eye)
- The choroid (the layer behind the retina that contains major blood vessels.
  The damage to the eyes is sometimes called retinochoroiditis and can cause eye problems including:
  - ✓ loss of eyesight
  - ✓ a squint (when one eye looks in a slightly different direction to the other one)
  - ✓ clouding of the eye lens (cataract)
  - ✓ eye shrinking (microphthalmia)
  - ✓ Loss of cells and tissue from the optic nerve, which connects the eye to the brain, resulting in poor vision (optic atrophy).
    Congenital toxoplasmosis:
    In most cases, babies born with congenital toxoplasmosis develop normally after treatment with antibiotics. However, in up to four per cent of cases, serious complications can develop within the first years of life. These include:
  - ✓ Death
  - ✓ Permanent brain damage
  - ✓ Permanent visual impairment (partial or complete loss of sight) in both eyes.

A common complication of congenital toxoplasmosis is retinochoroiditis. The risk of this occurring is around 10 per cent in infants born with congenital toxoplasmosis. One study found that the 18 per cent of children with congenital toxoplasmosis had at least one ocular lesion as a result of retinochoroiditis. Of these children, 42 per cent developed a second ocular lesion.

Cases of ocular toxoplasmosis can also occur years later. For example, one study of cases of congenital ocular toxoplasmosis found that the average age at which it appeared was nine years old.

It is also possible for someone to develop complications when they are in their twenties or thirties. These may include:

- ✓ Learning disabilities
- ✓ Hearing loss
- ✓ Ocular toxoplasmosis. [10]

Diagnosis

Physical examination:

The most common physical examination findings of toxoplasmosis include painless lymphadenopathy in immunocompetent individuals. Other findings include fever, malaise, myalgias, and a maculopapular skin rash that spares the palms and the soles. In retinochoroiditis examination reveals multiple yellow-white cotton like patches with indistinct margins located in small clusters in the posterior pole.

Vital signs:

- ✓ Fever
- ✓ Tachypnea

HEENT:

- ✓ Blurry vision

Skin:
Maculopapular rash that spares hands and soles
Lymph nodes:
Regional lymph node may be seen
Cardiovascular system:

- ✓ Regular rate and rhythm
- ✓ Normal S1,S2
- ✓ No murmurs, rubs, or gallops.

Lungs:
Findings consistent with parenchymal consolidation in cases of pulmonary pneumonitis such as:

- ✓ Dullness to percussion
- ✓ Increased fremitus
- ✓ Signs of pleura involvement such as pleuritic rub.

Abdominal:

- ✓ Abdomen soft-non distended with no scars or striations
- ✓ No pulsatile masses or abdominal bruits auscultated
- ✓ Hepatosplenomegaly in disseminated cases.

Neurological:
Normal examination finding unless the infection is disseminated to brain resulting in meningitis, then findings include:

- ✓ Altered level of consciousness
- ✓ Nuchal rigidity
- ✓ Unsteady gait
- ✓ Slurred speech
- ✓ Muscle weakness. [11]

Without specific screening, toxoplasmosis is often difficult to diagnose because signs and symptoms, when they occur, are similar to those of more common illnesses, such as the flu and mononucleosis.

Testing during pregnancy:

Pregnant ladies may have blood tests that check for antibodies to the parasite. Antibodies are proteins produced by immune system in response to the presence of foreign substances, such as parasites. Because these antibody tests can be difficult to interpret, all positive results be confirmed by a laboratory that specializes in diagnosing toxoplasmosis.

Testing baby for toxoplasmosis:

- ✓ Amniocentesis:

In this procedure, which may be done safely after 15 weeks of pregnancy, your doctor uses a fine needle to remove a small amount of fluid from the fluid-filled sac that surrounds the fetus (amniotic sac). Tests are then performed on the fluid to check for evidence of toxoplasmosis. Amniocentesis carries a slight risk of miscarriage and minor complications, such as cramping, leaking fluid or irritation where the needle was inserted.

- ✓ Ultrasound scan:

This test uses sound waves to produce images of your baby in the womb. A detailed ultrasound can't diagnose toxoplasmosis. It can however, show whether your baby has certain signs, such as fluid buildup in the brain (hydrocephalus). However, a negative ultrasound doesn't rule out the possibility of infection. For that reason, your newborn will need an examination and follow-up blood tests during the first year of life.

Testing in severe cases:

- ✓ Magnetic resonance imaging (MRI):

This test uses a magnetic field and radio (electromagnetic) waves to create cross-sectional images of your head and brain. During the procedure, you lie inside a large, doughnut-shaped machine that contains a magnet surrounded by coils that send and receive radio waves.

In response to the radio waves, body produces faint signals that are picked up by the coils and processed into images by a computer. MRI is noninvasive and poses minimal risks to your health.

- ✓ Brain biopsy.

In rare cases, especially if you don't respond to treatment, a neurosurgeon may take a small sample of brain tissue. The sample is then analyzed in a laboratory to check for toxoplasmosis cysts. [12]

Serological Tests:

Different serological tests often measure different antibodies that possess unique patterns of rise and fall with time after infection. A combination of serological tests is frequently required to establish whether an individual has been more likely infected in the distant past or has been recently infected.

An IgM test is used to help determine whether a patient has been infected recently or in the distant past. Because of the significant potential of misinterpreting a positive IgM test result,

confirmatory testing should be performed. Despite the wide distribution of commercial test kits to measure IgM antibodies, these kits often have low specificity and the reported results are frequently misinterpreted. In addition, IgM antibodies can persist for months to more than one year.

Toxoplasma Serological Profile (TSP):

For confirmatory testing, TSL-PAMFRI offers a panel of tests (the Toxoplasma Serological Profile (TSP) comprised of the Sabin-Feldman Dye Test (DT), double sandwich IgM ELISA, IgA ELISA, IgE ELISA, and AC/HS test. The TSP has been used successfully by our group to determine whether serological test results are more likely consistent with infection acquired in the recent or more distant past.

The TSP has been clinically helpful in the setting of toxoplasmic lymphadenitis, myocarditis, polymyositis, chorioretinitis, and during pregnancy. For sera with positive results in IgG and IgM tests, the discriminatory power of the TSP to differentiate between recently acquired and chronic infection is superior to any single serological test.

Recently, several tests for avidity of toxoplasma IgG antibodies have been introduced to help discriminate between recently acquired and distant infection. Studies of the kinetics of the avidity of IgG in pregnant women who have seroconverted during gestation have shown that women with high avidity test results were infected with T. gondii at least 3 to 5 months earlier (time to conversion from low to high avidity antibodies varies with the method used). Because low avidity antibodies may persist for many months, their presence does not necessarily indicate recently acquired infection.

IgG Antibodies:

Dye Test:

At TSL-PAMFRI IgG antibodies are primarily measured by the Sabin-Feldman Dye Test (DT). The DT is a sensitive and specific neutralization test in which live organisms are lysed in the presence of complement and the patient's IgG T. gondii -specific antibody. IgG antibodies usually appear within 1 to 2 weeks of the infection, peak within 1 to 2 months, fall at variable rates, and usually persist for life. The titer does not correlate with the severity of illness.

A positive DT establishes that the patient has been exposed to the parasite. A negative DT essentially rules out prior exposure to T. gondii (unless the patient is hypogammaglobulinemic). However, in a small number of patients, IgG antibodies might not be detected within 2 to 3 weeks after the initial exposure to the parasite. In addition, rare cases of toxoplasmic chorioretinitis and TE (toxoplasmic encephalitis) in immunocompromised patients have been documented in patients negative for T. gondii -specific IgG antibodies.

Differential agglutination (AC/HS):

The differential agglutination test (also known as the "AC/HS test") uses two antigen preparations that express antigenic determinants found early following acute infection (AC antigen) or in the later stages of infection (HS). Ratios of titers using AC versus HS antigens are interpreted as acute, equivocal, non-acute patterns of reactivity or non-reactive. The acute pattern may persist for one or more years following infection. This test has proved useful in helping differentiate acute from chronic infections but is best used in combination with a panel of other tests (e.g.: the TSP).

Avidity:

The functional affinity of specific IgG antibodies is initially low after primary antigenic challenge and increases during subsequent weeks and months. Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex. The avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated serum.

We routinely employ the avidity test as an additional confirmatory diagnostic tool in the TSP for those patients with a positive and/or equivocal IgM test or acute and/or equivocal pattern in the AC/HS test. Health care providers and clinical laboratories involved in the care of pregnant women should be aware that avidity testing is a confirmatory test and not the ultimate test for decision-making. Its highest value is observed when laboratory test results reveal high IgG avidity antibodies and the serum is obtained during the time window of exclusion of acute infection for a particular method (i.e. 12 weeks for the Labsystems method, 16 weeks for the VIDAS-bioMérieux method). Low or equivocal IgG avidity antibody results should not be interpreted as diagnostic of recently acquired infection. These low or equivocal avidity antibodies can persist for months to one year or longer.

IgM Antibodies:

IgM antibodies are measured by the "double-sandwich" or "immuno-capture" IgM-ELISA method. This method avoids false positive results due to the presence of rheumatoid factor and antinuclear antibodies.

In patients with recently acquired infection, IgM T. gondii antibodies are detected initially and, in most cases, these titers become negative within a few months. However, in some patients, positive IgM T.gondii -specific titers can be observed during the chronic stage of the infection. IgM antibodies have been reported to persist as long as 12 years after the acute infection. Persistence of these IgM antibodies does not appear to have any clinical relevance and these patients should be considered chronically infected.

The FDA has recommended that sera with positive IgM test results obtained at non-reference laboratories should be sent to a Toxoplasma reference laboratory. At our reference laboratory, these referred IgM positive sera undergo confirmatory testing and the results are interpreted as i)

a recently acquired infection, ii) an infection acquired in the distant past or iii) a false positive result.

IgA Antibodies:

IgA antibodies may be detected in sera of acutely infected adults and congenitally infected infants using ELISA or ISAGA methods. As is true for IgM antibodies to the parasite, IgA antibodies may persist for many months to more than one year. For this reason they are of little additional assistance for diagnosis of the acute infection in the adult. In contrast, the increased sensitivity of IgA assays over IgM assays for diagnosis of congenital toxoplasmosis represents an advance in diagnosis of the infection in the fetus and newborn. In a number of newborns with congenital toxoplasmosis and negative IgM antibodies, the serological diagnosis has been established by the presence of IgA and IgG antibodies.

IgE Antibodies:

IgE antibodies are detectable by ELISA in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmic chorioretinitis. The duration of IgE seropositivity is less than with IgM or IgA antibodies and hence appears useful as an adjunctive method for identifying recently acquired infections.

Molecular test:

Polymerase Chain Reaction (PCR):

PCR amplification is used to detect T. gondii DNA in body fluids and tissues. It has been successfully used to diagnose congenital, ocular, cerebral and disseminated toxoplasmosis. PCR performed on amniotic fluid has revolutionized the diagnosis of fetal T. gondii infection by enabling an early diagnosis to be made, thereby avoiding the use of more invasive procedures on the fetus. PCR has allowed detection of T. gondii DNA in brain tissue, cerebrospinal fluid (CSF), vitreous and aqueous fluid, bronchoalveolar lavage (BAL) fluid, urine, amniotic fluid and peripheral blood.

Histologic Diagnosis:

Demonstration of tachyzoites in tissue sections or smears of body fluid (e.g., CSF, amniotic fluid or BAL) establishes the diagnosis of the acute infection. It is often difficult to demonstrate tachyzoites in conventionally stained tissue sections. The immunoperoxidase technique, which uses antisera to T. gondii, has proven both sensitive and specific; it has been successfully used to demonstrate the presence of the parasite in the central nervous system of AIDS patients. The immunoperoxidase method is applicable to unfixed or formalin-fixed paraffin-embedded tissue sections.

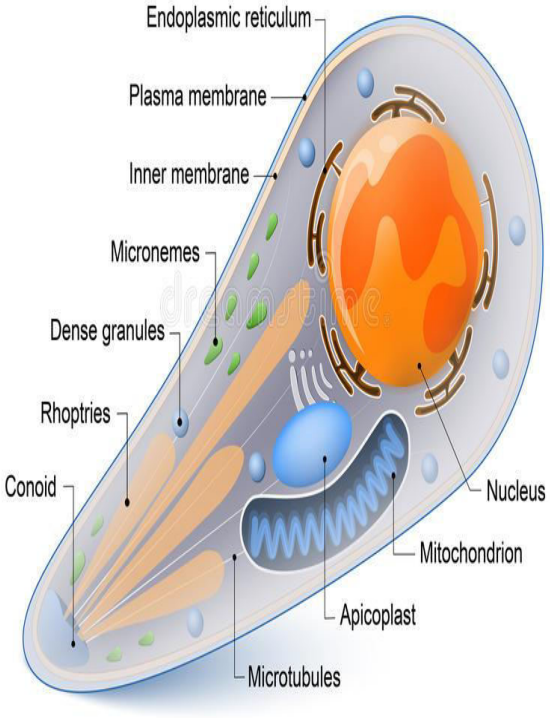
A rapid and technically simple method is the detection of T. gondii in air-dried, Wright-Giemsa-stained slides of centrifuged (e.g., cytocentrifuge) sediment of CSF or of brain aspirate or in impression smears of biopsy tissue. Our laboratory can assist in reviewing such preparations.

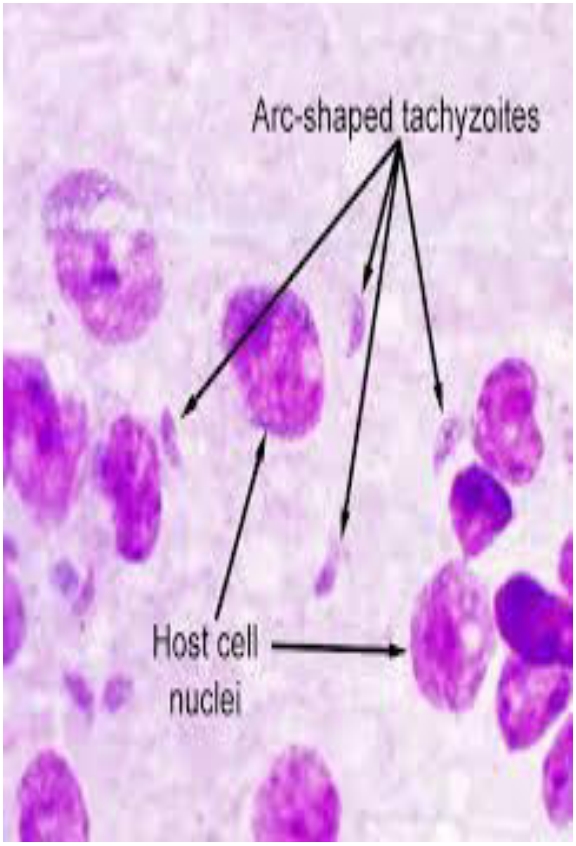
The presence of multiple tissue cysts near an inflammatory necrotic lesion probably establishes the diagnosis of acute infection or reactivation of latent infection.

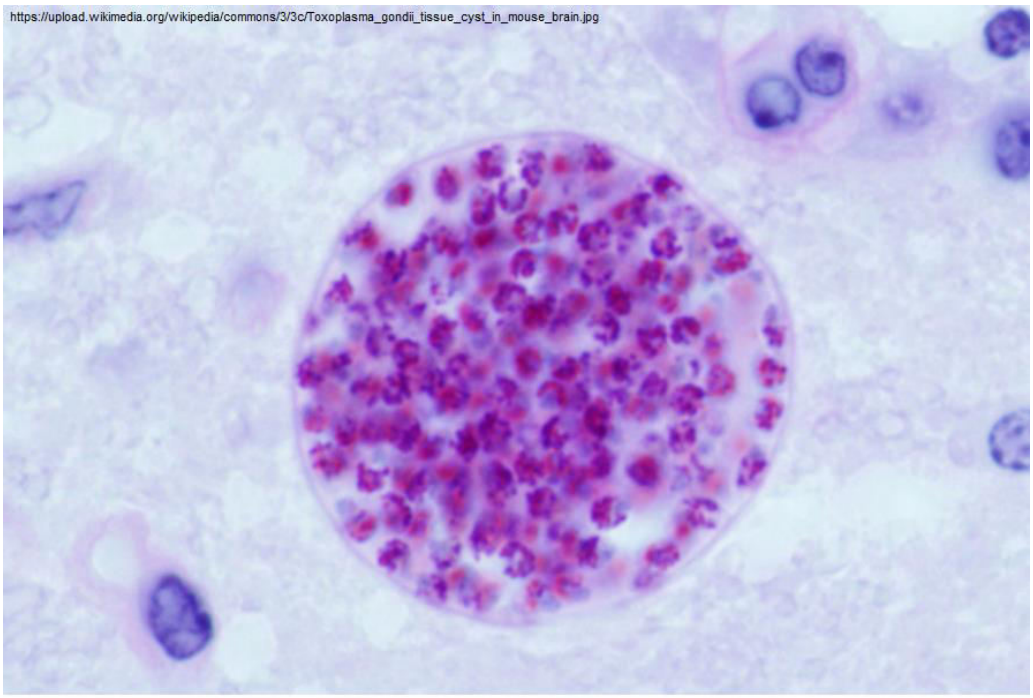
Isolation of T. gondii:

Isolation of T. gondii from blood or body fluids establishes that the infection is acute. Attempts at isolation of the parasite can be performed at TSL-PAMFRI by mouse inoculation. [13]

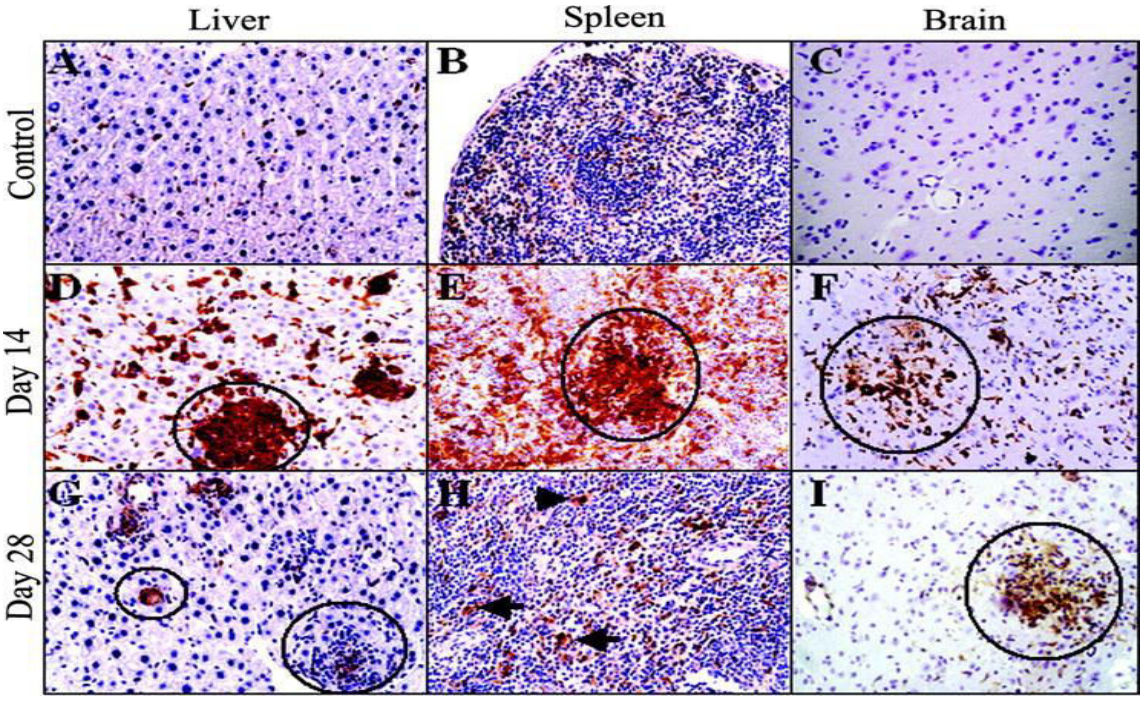
Toxoplasma gondii







A *T. Gondii* cyst within a mouse brain



Prevention & Control

Reduce Risk from Food:

To prevent risk of toxoplasmosis and other infections from food:

Cook food to safe temperatures. A food thermometer should be used to measure the internal temperature of cooked meat. Do not sample meat until it is cooked. USDA recommends the following for meat preparation.

For Whole Cuts of Meat (excluding poultry)

Cook to at least 145° F (63° C) as measured with a food thermometer placed in the thickest part of the meat, then allow the meat to rest* for three minutes before carving or consuming.

For Ground Meat (excluding poultry)

Cook to at least 160° F (71° C); ground meats do not require a rest* time.

For All Poultry (whole cuts and ground)

Cook to at least 165° F (74° C), and for whole poultry allow the meat to rest* for three minutes before carving or consuming.

*According to USDA, "A 'rest time' is the amount of time the product remains at the final temperature, after it has been removed from a grill, oven, or other heat source. During the three minutes after meat is removed from the heat source, its temperature remains constant or continues to rise, which destroys pathogens."

•Freeze meat for several days at sub-zero (0° F) temperatures before cooking to greatly reduce chance of infection.

•Peel or wash fruits and vegetables thoroughly before eating.

•Wash cutting boards, dishes, counters, utensils, and hands with hot soapy water after contact with raw meat, poultry, seafood, or unwashed fruits or vegetables.

Reduce Risk from the Environment

To prevent risk of toxoplasmosis from the environment:

•Avoid drinking untreated drinking water.

•Wear gloves when gardening and during any contact with soil or sand because it might be contaminated with cat feces that contain Toxoplasma. Wash hands with soap and warm water after gardening or contact with soil or sand.

•Teach children the importance of washing hands to prevent infection.

•Keep outdoor sandboxes covered.

•Feed cats only canned or dried commercial food or well-cooked table food, not raw or undercooked meats.

•Change the litter box daily if you own a cat. The Toxoplasma parasite does not become infectious until 1 to 5 days after it is shed in a cat's feces. If you are pregnant or immunocompromised:

1. Avoid changing cat litter if possible. If no one else can perform the task, wear disposable gloves and wash your hands with soap and warm water afterwards.
2. Keep cats indoors.
3. Do not adopt or handle stray cats, especially kittens. Do not get a new cat while you are pregnant. [14]

Treatment

Healthy people (nonpregnant):

Most healthy people recover from toxoplasmosis without treatment. Persons who are ill can be treated with a combination of drugs such as pyrimethamine and sulfadiazine, plus folinic acid.

Pregnant women, newborns, and infants:

Pregnant women, newborns, and infants can be treated, although the parasite is not eliminated completely. The parasites can remain within tissue cells in a less active phase; their location makes it difficult for the medication to completely eliminate them.

Persons with ocular disease:

Persons with ocular toxoplasmosis are sometimes prescribed medicine to treat active disease by their ophthalmologist. Whether or not medication is recommended depends on the size of the eye lesion, the location, and the characteristics of the lesion (acute active, versus chronic not progressing.

Persons with compromised immune systems:

Persons with compromised immune systems need to be treated until they have improvement in their condition. For AIDS patients, continuation of medication for the rest of their lives may be necessary, or for as long as they are immunosuppressed. [(15)]

Currently recommended drugs in the treatment of toxoplasmosis act primarily against the tachyzoite form of T gondii; thus, they do not eradicate the encysted form (bradyzoite). Pyrimethamine is the most effective agent and is included in most drug regimens. Leucovorin (i.e., folinic acid) should be administered concomitantly to prevent bone marrow suppression. Unless circumstances preclude using more than 1 drug, a second drug (e.g., sulfadiazine, clindamycin) should be added.

The efficacy of azithromycin, clarithromycin, atovaquone, dapsone, and cotrimoxazole is unclear; therefore, they should be used only as alternatives in combination with pyrimethamine. The most effective available therapeutic combination is pyrimethamine plus sulfadiazine or trisulfapyrimidines (e.g., a combination of sulfamerazine, sulfamethazine, and sulfapyrazine). These agents are active against tachyzoites and are synergistic when used in combination.

Careful attention to dosing regimen is necessary because it differs depending on patient variables (e.g., immune status, pregnancy). Pyrimethamine may be used with sulfonamides, quinine, and other antimalarials and with other antibiotics.

Nonpregnant patients:

Immunocompetent, nonpregnant patients typically do not require treatment. Treatment of nonpregnant patients is described below.

The 6-week regimen is as follows:

• Pyrimethamine (100mg loading dose orally followed by 25-50 mg/day) plus sulfadiazine (2-4 g/day divided 4 times daily) OR

• Pyrimethamine (100-mg loading dose orally followed by 25-50 mg/day) plus clindamycin (300 mg orally 4 times daily)

• Folinic acid (leucovorin) (10-25 mg/day) should be given to all patients to prevent hematologic toxicity of pyrimethamine

• Trimethoprim (10 mg/kg/day) sulfamethoxazole (50 mg/kg/day) for 4 weeks

Sulfadiazine or clindamycin can be substituted for azithromycin 500 mg daily or atovaquone 750 mg twice daily in immunocompetent patients or in patients with a history of allergy to the former drugs

Consider steroids in patients with radiologic midline shift, clinical deterioration after 48 hours, or elevated intracranial pressure.

Pregnant patients:

The diagnosis of acute infection is often difficult to make during pregnancy, and the administration of empiric antimicrobial therapy is discouraged.

Substantial controversy exists regarding the efficacy of treatment during pregnancy in terms of reducing the risk of fetal exposure and the subsequent development of clinical disease such as retinochoroiditis or CNS abnormalities.

Controversy also exists regarding the optimal regimen for treating maternally acquired infection. Spiramycin and pyrimethamine-sulfonamide are used, but given the infrequency of fetal infection and the asymptomatic nature of most fetal infections, treatment effects are difficult to measure. Spiramycin appears to be somewhat more easily tolerated than pyrimethamine-sulfonamide.

A dosing regimen for pregnant patients is as follows:

Spiramycin 1 g orally every 8 hours

• If the amniotic fluid test result for T gondii is positive: 3 weeks of pyrimethamine (50 mg/day orally) and sulfadiazine (3 g/day orally in 2-3 divided doses) alternating with a 3-week course of spiramycin 1 g 3 times daily for maternal treatment OR

• Pyrimethamine (25 mg/day orally) and sulfadiazine (4 g/day orally) divided 2 or 4 times daily until delivery (this agent may be associated with marrow suppression and pancytopenia) AND

• Leucovorin 10-25 mg/day orally to prevent bone marrow suppression

Patients with AIDS:

Patients with AIDS are treated with pyrimethamine 200 mg orally initially, followed by 50-75 mg/day orally plus folinic acid 10 mg/day orally plus sulfadiazine 4-8 g/day orally for as long as 6 weeks, followed by lifelong suppressive therapy or until immune reconstitution.

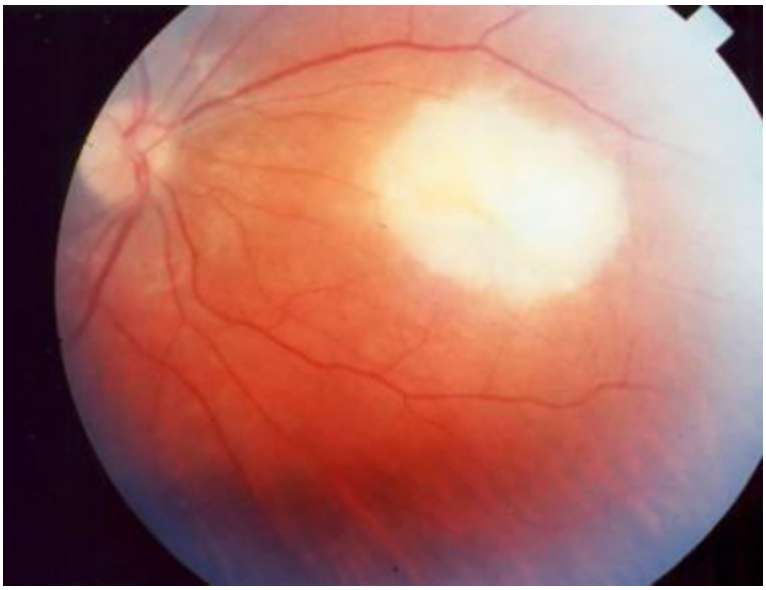
Suppressive therapy for patients with AIDS (CD4 count < 100 cells/µL) is pyrimethamine 50mg/day orally plus sulfadiazine 1-1.5 g/day orally plus folinic acid 10 mg/day orally for life or until immune reconstitution.

Patients with AIDS, CNS toxoplasmosis, and evidence of midline shift or increased intracranial pressure may also benefit from steroid therapy.

Diagnosing toxoplasmosis in the absence of definitive tissue or culture evidence may be perilous because serology may be misleading and a false-positive IgM result is somewhat common. Consequently, empiric therapy should be avoided.

Retinitis:

The mere presence of a focus of retinitis is not always an indication for treatment. Small, peripheral lesions generally heal spontaneously and may be followed conservatively. On the other hand, lesions in the vascular arcade, lesions near the optic disc (Jensen papillitis), lesions in the papillomacular bundle, or large lesions (irrespective of location) are treated. Patients with severe, debilitating vitreitis are also treated aggressively. (See the image below.)



Acute macular retinitis associated with primary acquired toxoplasmosis, requiring immediate systemic therapy.

In a prospective trial, treatment with several regimens failed to shorten the duration of inflammatory activity or to prevent recurrences. However, treatment did reduce the size of the ultimate retinochoroidal scar.

In addition, experts differ on their preferred initial treatment. In a report, one third of respondents preferred triple therapy (i.e., pyrimethamine, sulfadiazine, prednisone), and a little more than one quarter of respondents preferred quadruple therapy (i.e., pyrimethamine, sulfadiazine, clindamycin, prednisone). [16]

Toxoplasmosis medications:

- **Daraprim (pyrimethamine)**:

✓ Name of the medicinal product:
Daraprim 25 mg Tablets

✓ Qualitative and quantitative composition:
Each tablet contains 25 mg of pyrimethamine.

✓ Pharmaceutical form:

Tablet.
Each tablet is white and round with the marking GS A3A.

✓ Clinical particulars:

- Therapeutic indications:
  Toxoplasmosis, including ocular infections, proven foetal infection following maternal infection during pregnancy, and toxoplasmosis in immune-deficient patients (for the treatment of toxoplasmosis Daraprim must always be used in combination with a synergistic agent e.g. sulphadiazine).
  Treatment is not normally required for asymptomatic or mild toxoplasma infection.

- Posology and method of administration:
  Posology:
  Toxoplasmosis (including ocular infections):
  Daraprim should be given concurrently with sulphadiazine or another appropriate antibiotic.

In the treatment of toxoplasmosis, all patients receiving Daraprim should be given a folinic acid supplement (calcium folinate) to reduce the risk of bone marrow depression.
Daraprim treatment should generally be given for 3 to 6 weeks and not less than six weeks in immunosuppressed patients. If further therapy is indicated, a period of two weeks should elapse between treatments.
Adults:

A loading dose of Daraprim 100 mg should be given for the first 1 to 2 days, followed by 25 mg to 50 mg daily. This should be given together with 2 g to 4 g of sulphadiazine daily in divided doses.

- Foetal toxoplasmosis during pregnancy:
  Daraprim 50 mg every 12 hours for 2 days, followed by 50 mg daily. This should be given together with an initial dose of sulfadiazine 75 mg/kg, followed by 50 mg/kg every 12 hours (to a maximum of 4 g daily).
  Immune-deficient adults and adolescents:

Guidelines for the treatment of opportunistic infections in HIV-infected adults and adolescents consider pyrimethamine plus sulfadiazine to be the initial therapy of choice for Toxoplasma gondii encephalitis and recommend the following doses, based on body-weight, be given for at least 6 weeks:
- less than 60 kg - pyrimethamine 200 mg orally, followed by 50 mg daily plus sulfadiazine 1 g orally every 6 hours.
- 60 kg or more - pyrimethamine 200 mg orally, followed by 75 mg daily plus sulfadiazine 1.5 g orally every 6 hours.

Pediatric Population:
Children over 6 years:
A loading dose of Daraprim 100 mg should be given for the first 1 to 2 days, followed by 25 mg to 50 mg daily. This should be given together with 2 g to 4 g of sulphadiazine daily in divided doses.
Children aged 5 to 6 years:

An initial dose of Daraprim 2 mg/kg bodyweight (to a maximum of 50 mg) followed by 1 mg/kg bodyweight/day (to a maximum of 25 mg); combined with sulphadiazine 150 mg/kg bodyweight (maximum 2 g) daily in four divided doses.
Immune-deficient children:
Dosage regimens for immune-deficient children are not defined.

Children under 5 years:
There is insufficient data to provide specific dose recommendations in children. This formulation is not suitable for children under 5 years.
Elderly:
There is no definitive information on the effect of Daraprim on elderly individuals. It is theoretically possible that elderly patients might be more susceptible to folate depression associated with the daily administration of Daraprim in the treatment of toxoplasmosis, and supplementation of folinic acid is therefore essential.
Patients with renal impairment:

Daraprim should be given with caution to patients with renal impairment. Since Daraprim is co-administered with a sulphonamide care should be taken to avoid accumulation of the sulphonamide in patients with renal impairment.
Patients with hepatic impairment:
Daraprim should be given with caution to patients with hepatic impairment. There are no general recommendations for dosage reductions for liver-impaired states but consideration should be given to dose adjustments for individual cases.
Method of administration:
For oral administration.

- Contraindications:
  Daraprim is contraindicated in:
  Hypersensitivity to pyrimethamine or to any of the excipients of this medicinal product.
  Daraprim should not generally be used during the first trimester of pregnancy.
  Since Daraprim is to be taken in conjunction with another drug for the indications listed, the relevant prescribing information for the synergistic agent should also be considered.
  Breast-feeding should be avoided during toxoplasmosis treatment.
    - Special warnings and precautions for use:
      Depression of hematopoiesis:
      Daily therapeutic doses of Daraprim have been shown to depress hematopoiesis in 25% to 50% of patients. The likelihood of inducing leucopenia, anaemia or thrombocytopenia is reduced by concurrent administration of calcium folinate. Pancytopenia, responsive to folate, has been reported in patients with probable pre-existing folate deficiency. Fatalities have occurred in the absence of folate treatment.
      Prevention of haematological toxicity:
      During pregnancy and in other conditions predisposing to folate deficiency, a folate supplement should be given. The co-administration of a folate supplement is necessary for treatment of toxoplasmosis. Full blood counts should be carried out weekly during therapy and for a further two weeks after treatment is stopped. In immunosuppressed patients, full blood counts should be carried out twice weekly. Should signs of folate deficiency develop, treatment must be discontinued and high doses of calcium folinate administered. Calcium folinate should be used because folic acid does not correct folate deficiency due to dihydrofolate reductase inhibitors.
      Daraprim may exacerbate folate deficiency in subjects predisposed to this condition through disease or malnutrition. Accordingly, a calcium folinate supplement should be given to

such individuals. In patients with megaloblastic anaemia due to folate deficiency the risks versus benefits of administering Daraprim require careful consideration.

Seizures:

Caution should be exercised in administering Daraprim to patients with a history of seizures; large loading doses should be avoided in such patients.

Risk of crystalluria:

When a sulphonamide is given an adequate fluid intake should be ensured to minimize the risk of crystalluria.

Precautions applicable to sulphonamides:

Since Daraprim is administered with a sulphonamide for the conditions indicated the general precautions applicable to sulphonamides should be observed.

Renal impairment:

The kidney is not the major route of excretion of pyrimethamine and excretion is not significantly altered in patients with renal failure. There are, however, no substantial data on the use of Daraprim in patients with renal impairment, therefore Daraprim should be given with caution. Since Daraprim is co-administered with a sulphonamide, care should be taken to avoid accumulation of the sulphonamide in renally impaired patients.

Hepatic impairment:

The liver is the main route for metabolism of pyrimethamine. Data on the use of Daraprim in patients with liver disease are limited. Daraprim should be given with caution to patients with hepatic impairment. There are no general recommendations for dosage reductions for liver-impaired states but consideration should be given to dose adjustment for individual cases.

Lactose:

This medicinal product contains lactose. Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicine.

- Interaction with other medicinal products and other forms of interaction:

  Folate inhibitors, agents associated with myelosuppression.

  Daraprim, by its mode of action, may further depress folate metabolism in patients receiving treatment with other folate inhibitors, or agents associated with myelosuppression, including cotrimoxazole, trimethoprim, proguanil, zidovudine, or cytostatic agents (e.g. methotrexate).

  Cases of fatal bone marrow aplasia have been associated with the administration of daunorubicin, cytosine arabinoside and pyrimethamine to individuals suffering from acute myeloid leukemia.

Megaloblastic anaemia has been reported occasionally in individuals who took pyrimethamine concurrently with a trimethoprim/sulphonamide combination.
Methotrexate:
Convulsions have occurred after concurrent administration of methotrexate and pyrimethamine to children with central nervous system leukemia.
Other antimalarial drugs:
Seizures have occasionally been reported when pyrimethamine was used in combination with other antimalarial drugs.
Lorazepam:
The concurrent administration of lorazepam and Daraprim may induce hepatotoxicity.
Antacid salts, kaolin:
In vitro data suggest that antacid salts and the anti-diarrheal agent kaolin reduce the absorption of pyrimethamine.
Highly protein bound compounds:
The high protein binding exhibited by pyrimethamine may prevent protein binding by other compounds (e.g. quinine or warfarin). This could affect the efficacy or toxicity of the concomitant drug depending on the levels of unbound drug.

- Fertility, pregnancy and lactation:
  Pregnancy:
  Daraprim should not be used during the first trimester of pregnancy unless the benefits outweigh the risk. Daraprim has been shown to be teratogenic in animal studies. The risks associated with the administration of Daraprim must be balanced against the dangers of abortion or foetal malformation due to the infection.
  Treatment with Daraprim and sulfadiazine during pregnancy is indicated in the presence of confirmed placental or foetal infection or when the mother is at risk of serious sequelae. However, in view of the theoretical risk of foetal abnormality arising from the use of Daraprim in early pregnancy, its use in combination therapy should be restricted to the second and third trimesters.
  Pregnant women receiving Daraprim must be given a concurrent folinic acid supplement.
  Breastfeeding:
  Pyrimethamine enters human breast milk. It has been estimated that over a 9-day period an average weight infant would receive about 45% of the dose ingested by the mother. In view of the high doses of pyrimethamine and concurrent sulphonamides

needed in toxoplasmosis treatment, breast feeding should be avoided for the duration of treatment.
Fertility:
There are no relevant data available.

- Effects on ability to drive and use machines:
  No studies on the effects on the ability to drive and use machines have been performed. Some patients may experience dizziness or convulsions, therefore, caution is recommended.
- Undesirable effects:
  Since a concurrent sulphonamide is to be taken with pyrimethamine for the indications listed, the relevant prescribing information for the sulphonamide should be consulted for sulphonamide-associated adverse events.
  It is important to note that the frequency categories assigned for each adverse event below are only estimates as suitable data for accurately calculating incidence were not available. Adverse events may vary in their incidence according to the indication and the possible contribution of concomitant sulphonamides to the occurrence of these events is unknown. In addition some events may be related to the underlying disease.
- Overdose:
  Symptoms:
  Vomiting and convulsions occur in cases of severe, acute overdoses. Ataxia, tremor and respiratory depression can also occur. There have been isolated cases with fatal outcomes following acute overdose of pyrimethamine.
  Chronic excess doses can result in bone marrow depression (e.g. megaloblastic anaemia, leucopenia, thrombocytopenia) resulting from folic acid deficiency.
  Management:
  Routine supportive treatment, including maintenance of a clear airway and control of convulsions.
  Adequate fluids should be given to ensure optimal diuresis.
  To counteract possible folate deficiency, calcium folinate should be given until signs of toxicity have subsided. There may a delay of 7 to 10 days before the full leucopenic side effects become evident, therefore calcium folinate therapy should be continued for the period at risk.

✓ Pharmacological properties:

- Pharmacodynamic properties
  Pyrimethamine is an antiparasitic agent.
  Pharmacotherapeutic group: diaminopyrimidines, ATC code: P01B D01.

Mechanism of Action:
The antiparasitic action of pyrimethamine is due to its specific activity on folic acid metabolism in the Plasmodium and Toxoplasma parasites. In this respect it competitively inhibits the dihydrofolate reductase enzyme with an affinity far greater for the protozoal than for the human enzyme.

- Pharmacokinetic properties:
  Absorption:
  Pyrimethamine is almost completely absorbed from the gastrointestinal tract. Peak plasma concentrations generally occur 2 to 4 hours after a dose and can vary widely between individuals; concentrations ranging from 260 to 1411 ng/ml after daily oral doses of 25 mg.
  Distribution:
  The volume of distribution for pyrimethamine is approximately 2L/kg. In patients with HIV infection, population pharmacokinetic analysis has indicated that the mean volume of distribution (corrected for bioavailability) is 246+/-64L.
  About 80 to 90% of the pyrimethamine is bound to plasma proteins.
  Pyrimethamine is mainly concentrated in the kidneys, lungs, liver, and spleen. In AIDS patients given daily dose of pyrimethamine, concentrations of about one-fifth of those in the serum occur in cerebrospinal fluid.
  Pyrimethamine crosses the placenta. It is distributed into breast milk.
  Elimination:
  Pyrimethamine is predominantly metabolized by the liver. The mean elimination half-life is 85 hours. Pyrimethamine is slowly excreted in urine. In AIDS patients, the total clearance is 1.28+/-0.41L/h resulting in an elimination half-life of 139+/-34h. Data are lacking on the nature of the metabolites of pyrimethamine, their route/rate of formation and elimination in man and any pharmacological activity, particularly after prolonged daily dosing.
  Multiple dose studies indicate that steady state is achieved in 12 to 20 days with daily dosing. It is theoretically possible that metabolic pathways might be saturable, leading to excessive accumulation of the drug in some patients. However, it has been demonstrated that plasma levels are approximately proportional to dose at steady state so this appears unlikely. Genetic variation in the exposure to pyrimethamine has been reported but these data are unsubstantiated.

Some studies in patients with AIDS have indicated shorter half-lives than those noted above: these are very likely to be a consequence of inappropriate sampling and analytical techniques. However, if there are patients in whom the half-life is particularly short, steady state therapeutic levels might be inadequate.

- Preclinical safety data:
  Mutagenicity:
  In microbial tests, pyrimethamine was found to be non-mutagenic in the Ames Salmonella assay whereas DNA damage was seen in the Escherichia coli repair assay. Further in vitro data indicate that pyrimethamine induces mutagenic activity in mouse lymphoma cells in the absence, but not in the presence of metabolic activation.
  Pyrimethamine also showed clastogenic activity in mammalian lymphocytes in the absence of metabolic activation.
  Following intraperitoneal administration, pyrimethamine has been shown to induce chromosomal damage in male rodent germ cells although studies in somatic cells (micronucleus tests) are either negative or inconclusive. Studies following oral administration of pyrimethamine in rodents showed negative results in female germ cells and in male and female bone marrow/peripheral blood cells.
  Carcinogenicity:
  A study in mice (dosed with either 500 or 1000 ppm pyrimethamine in the diet for 5 days per week, for 78 weeks) showed no evidence of carcinogenicity in females. Survival in the male mice did not allow for an assessment of carcinogenicity in this sex.
  A similar study in rats dosed at 200 or 400 ppm pyrimethamine showed no evidence of carcinogenicity.
  Teratogenicity:
  No changes in early development were seen in embryos from 15 mice given a single intra-gastric dose of pyrimethamine (50 mg/kg bodyweight) on the first day of gestation. However development of mouse and rat embryos in culture was severely hindered by pyrimethamine in a dose-dependent manner.
  Pyrimethamine was teratogenic in rodents and in the Gottingen minipig in a dose-dependent manner.
  Other studies in rats dosed at either 1 mg/kg or 10 mg/kg bodyweight showed some inhibition of developmental processes but no teratological effects.

Pyrimethamine was not teratogenic in rabbits at dose levels up to 100 mg/kg bodyweight/day administered on days 6 to 18 of pregnancy. Pyrimethamine markedly reduced early stage cell division in rabbit embryos but implantation and foetal development were normal.
Fertility:
A study in rats dosed with 5 mg/kg bodyweight/day for 6 weeks resulted in reduced sperm concentrations and testis weights, but there were no effects on fertility. Reversible arrest of spermatogenesis was shown in a study on mice dosed with 200 mg/kg/day for 50 days. However, this dose is far in excess of human therapeutic doses. [17]

- **Sulfadiazine:**

Group: antiprotozoal agent
Tablet 500 mg
General information:
An inhibitor of folic acid metabolism in some bacteria and protozoa that acts synergistically with pyrimethamine. It has been selected on pharmacokinetic grounds as an appropriate sulfonamide to use in combination with pyrimethamine in the treatment of toxoplasmosis.
Sulfadiazine is rapidly absorbed from the gastrointestinal tract and widely distributed in the body. The serum half-life is 10-12 hours. After partial acetylation in the liver it is excreted in the urine.
Clinical information:
Uses:
Treatment of toxoplasmosis in:

- ✓ Pregnant women after the first trimester when there is a danger of congenital transmission
- ✓ Infected neonates
- ✓ Infants, children and adults with chorioretinitis
- ✓ Active toxoplasmosis in patients who are immunodeficient as a consequence of drug treatment or disease.

Dosage and administration:

Sulfadiazine is always administered in this context orally in combination with pyrimethamine.

Pregnant women: 3 g daily in four divided doses.

Neonates: 85 mg/kg daily in two divided doses.

Chorioretinitis in adults: 2 g daily in four divided doses.

Immunodeficient patients: 4-6 g daily in four divided doses for at least 6 weeks followed by a suppressive dose of 2-4 g daily indefinitely.

Contraindications:

- ✓ Known hypersensitivity.
- ✓ Severe hepatic or renal dysfunction.
- ✓ Pregnancy during the first trimester

Precautions:

The blood count should be monitored twice weekly throughout therapy to detect signs of bone-marrow depression.

Any patient suspected of being sensitive to sulfonamides should never receive them again. Presumptive signs include skin rashes and evidence of hemolysis such as dark urine and purpura.

Sulfadiazine is less soluble in urine than many other sulfonamides. High urinary output must be maintained to avoid crystallization. Patients should be advised to drink 1.0-1.5 litres of alkaline water daily.

Concomitant administration of other drugs that interfere with folic acid metabolism (other than pyrimethamine) should be avoided whenever possible.

Use in pregnancy:

Sulfadiazine is contraindicated during the first trimester but may be given thereafter when there is a danger of congenital transmission.

Administration of sulfonamides can induce severe hypersensitivity reactions in the mother. Their action in displacing bilirubin from protein-binding sites has given rise to concern, based on data derived from premature neonates, that they may promote kernicterus. Although they readily cross the placental barrier there is no conclusive evidence that the fetus is at risk.

Adverse effects:

- ✓ Nausea, vomiting, diarrhoea and headache sometimes occur.
- ✓ Sulfonamide-induced hypersensitivity reactions, although uncommon, can be severe. They include rare life-threatening cutaneous reactions such as erythema multiforme (Stevens-Johnson syndrome) and toxic epidermal necrolysis.
- ✓ Crystalluria may result in dysuria, renal colic, haematuria and acute renal obstruction.
- ✓ Other infrequent reactions include granulocytopenia, agranulocytosis, aplastic anaemia, thrombocytopenic purpura and toxic hepatitis. Occasionally, hemolysis may occur in individuals deficient in glucose-6-phosphate dehydrogenase.

  Overdosage:

  Continuous forced diuresis may be beneficial and an alkaline urine should be maintained. Treatment is otherwise symptomatic.

  Storage:

  Tablets should be kept in well-closed containers, protected from light. [18]

Other medications for toxoplasmosis includes:

- **Clindamycin**
- **Azithromycin**
- **Sulfamethoxazole/trimethoprim**
- **Zithromax**
- **Leucovorin**
- **Co-trimoxazole**
- **Bactrim**
- **Mepron**
- **Clarithromycin**
- **Cleocin**
- **Biaxin**
- **Zmax**
- **Atovaquone**
- **Septra**
- **Sulfatrim**
- **Wellcovorin**
- **Cleocin phosphate**
- **Cleocin pediatric**
- **Cleocin HCL**
- **SMZ-TMP DS**. [19]

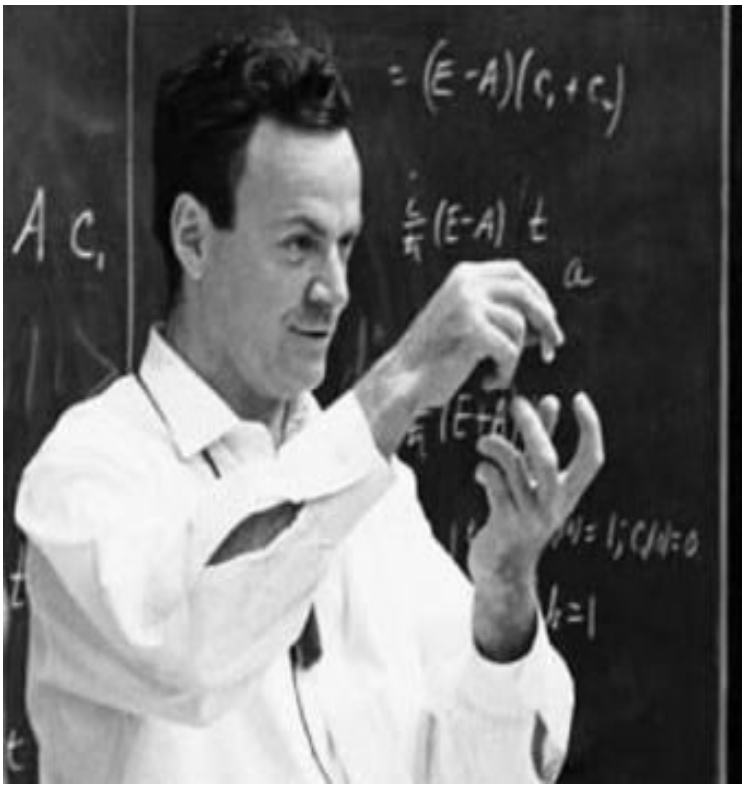
Role of nanotechnology in treatment of toxoplasmosis:

Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers.

Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering.

History:

The ideas and concepts behind nanoscience and nanotechnology started with a talk entitled "There's Plenty of Room at the Bottom" by physicist Richard Feynman at an American Physical Society meeting at the California Institute of Technology (CalTech) on December 29, 1959, long before the term nanotechnology was used. In his talk, Feynman described a process in which scientists would be able to manipulate and control individual atoms and molecules. Over a decade later, in his explorations of ultraprecision machining, Professor Norio Taniguchi coined the term nanotechnology. It wasn't until 1981, with the development of the scanning tunneling microscope that could "see" individual atoms that modern nanotechnology began. [20]



Founder K. Eric Drexler introduced the term "nanotechnology" to the world in 1986, using it to describe a 'bottom-up' approach. Drexler approaches nanotechnology from the point-of-view of a physicist, and defines the term as "large-scale mechanosynthesis based on positional control of chemically reactive molecules.

Basics of nanotechnology:

It uses a basic unit of measure called a "nanometer" (abbreviated nm). Derived from the Greek word for midget, "nano" is a metric prefix and indicates a billionth part (10-9).

There are one billion nm to a meter. Each nm is only three to five atoms wide. They're small. Really small. ~40,000 times smaller than the width of an average human hair.

A good reference to visit to help you understand the nanoscale materials end of "nanotech" is the Teacher's Guide To the (Small) World of Nanostructured Materials

RICHARD FEYNMAN

Richard P. Feynman was born in New York City on the 11th May 1918. He studied at the Massachusetts Institute of Technology where he obtained his B.Sc. in 1939 and at Princeton University where he obtained his Ph.D. in 1942. He was Research Assistant at Princeton (1940-1941), Professor of Theoretical Physics at Cornell University (1945-1950), and Visiting Professor and thereafter appointed Professor of Theoretical Physics at the California Institute of Technology (1950-1959). At present he is Richard Chace Tolman Professor of Theoretical Physics at the California Institute of Technology.

Professor Feynman is a member of the American Physical Society, the American Association for the Advancement of Science; the National Academy of Science; in 1965 he was elected a foreign member of the Royal Society, London (Great Britain)

Regarded as the father of nanotechnology. [21]

One aspect of nanotechnology is all about building working mechanisms using components with nanoscale dimensions (MNT), such as super small computers (think bacteria-sized) with today's MIPS capacity, or supercomputers the size of a sugar cube, possessing the power of a billion laptops, or a regular sized desktop model with the power of trillions of today's PC's.

The other aspect deals with scaling down existing technologies to the nanoscale, examples of which can be seen at our Current Uses page.

Some of the most promising potential of nanotechnology exists due to the laws of quantum physics. Quantum physics laws take over at this scale, enabling novel applications in optics, electronics, magnetic storage, computing, catalysts, and other areas.

Regardless of the diverse opinions on the rate at which nanotechnology will be implemented, people who make it a habit of keeping up with technology advances agree on this: it is a technology in its infancy, and it holds the potential to change everything.

Read this great Introduction from the Center for Responsible Nanotechnology for a better understanding of what nanotechnology is and is not, the social and business implications, and some steps being considered to control misuse.

Related and interwoven fields include, but are not limited to: Nanomaterials, Nanomedicine, Nanobiotechnology, Nanolithography, Nanoelectronics, Nanomagnetics, Nanorobots, Biodevices (biomolecular machinery), AI, MEMS (MicroElectroMechanical Systems), NEMS (NanoElectroMechanical Systems), Biomimetic Materials, Microencapsulation, and many others. [22]

- **Inorganic nanoparticles:**

Recent studies showed that; anti-Toxoplasma gondii potential of gold, silver, and platinum nanoparticles (NPs). Inorganic NPs (0.01–1,000 μg/mL) were screened for antiparasitic activity. The NPs caused >90% inhibition of T. gondii growth with EC50 values of ≤7, ≤1, and ≤100 μg/mL for gold, silver, and platinum NPs, respectively. The NPs showed no host cell cytotoxicity at the effective anti-T. Gondii concentrations; the estimated selectivity index revealed a ≥20-fold activity toward the parasite versus the host cell. [23]

Immunity to toxoplasmosis

Invasion process and intracellular niche:

The mechanisms by which T. gondii invades host cells and forms an intracellular niche have been extensively reviewed elsewhere, but several aspects of this process are directly relevant to immunity and pathogenesis. During invasion, three successive waves of proteins are secreted from parasite organelles, called the micronemes, dense granules, and rhoptries, into the host cell. These proteins can alter host cell function and inhibit the immune response directed towards the parasite. They also serve to modify the lipid membrane surrounding the parasite, forming a specialized intracellular organelle called the parasitophorous vacuole (PV). The PV allows for the transport of essential nutrients from the host cell to

the parasite, while preventing lysosomal fusion, which would lead to the killing of the parasite. The sequestered nature of the parasite within the PV raises several fundamental questions regarding the mechanisms by which the parasite interacts with the immune system. For example, can host cells sense the invading parasite, and how would infected cells access parasite antigens for presentation to T cells as is required for the effective control of the parasite.

Parasite virulence:

As is the case for many pathogens, the outcome of infection with T. gondii is highly dependent on the interplay of host and microbial factors. Genotypic studies have identified three lineages of T. gondii into which most strains found in North America and Western Europe can be broadly classified. In mouse models, parasites belonging to the Type I lineage are highly virulent whereas the Type II and Type III lineages are considered avirulent. These differences are also reflected in human disease, as ocular toxoplasmosis in humans is associated with Type I, but not Type II or Type III strains. Given the lethality of Type I strains during murine infection, the vast majority of insights into the mechanisms by which the host immune response controls infection have been gained through studies using avirulent isolates. However, the use of reverse genetics to compare parasite strains that differ in virulence has allowed the identification of secreted T. gondii kinases that modify host cell function. Understanding how these parasite enzymes impact host anti-microbial mechanisms provides one approach to define the events that determine the outcome of infection.

Innate immune responses to T. gondii:
Following challenge with T. gondii, monocytes, neutrophils and dendritic cells (DCs) are recruited to the site of infection, and all of these cell types have been implicated in resistance to this organism. However, questions remain about their specific roles in controlling infection. One of the most critical functions of the innate immune response to T. gondii is the ability to sense the pathogen and produce the cytokine IL-12, which stimulates natural killer (NK) cells and T cells to produce the cytokine Interferon-gamma (IFN-γ). IFN-γ is the major mediator of resistance to T. gondii and promotes multiple intracellular mechanisms to kill the parasite and inhibit its replication. This Th1 immune response, defined by the production of IL-12 and IFN-γ, is characteristic of infection with many intracellular pathogens, and as is the case for infection with other intracellular pathogens, mice deficient in either IL-12 or IFN-γ that are infected with T. gondii succumb to acute disease and demonstrate an inability to control parasite burden.

The innate production of IL-12 during toxoplasmosis requires that the parasite first be sensed by the host. Innate immune receptors called Toll Like Receptors

(TLRs) appear to have a role in this process. Thus, mice deficient in the adapter molecule MyD88, which is required for downstream signaling from most TLRs, are acutely susceptible to toxoplasmosis. Specific TLRs implicated in the immune response to T. gondii include TLRs 2, 4, 9 and 11. TLR11 responds to a profilin-like molecule conserved among protozoan parasites whereas TLRs 2 and 4 appear to recognize glycosylphosphatidylinositols on the surface of the parasite. Additionally, following oral infection with T. gondii, bacterial antigens translocate from the gut, and TLRs 2, 4, and 9 respond to these microbial insults, thus contributing to the development of the Th1 immune response. Although deficiency in any single TLR (of those tested to date) does not result in acute susceptibility to T. gondii, the relative contribution of each of these TLRs is illustrated by the increased cyst burden present in infected mice deficient in one or more of these receptors. Despite the critical importance of MyD88, other mechanisms of sensing the parasite clearly exist, as protective immunity can be induced in MyD88-deficient mice using a vaccine strain of the parasite, and IL-12 responses are not completely abolished in the absence of MyD88.

The relative contribution of DCs to the production of IL-12 during toxoplasmosis has been examined using two mouse models: one in which DCs can be selectively depleted, and another in which DCs specifically lack expression of MyD88. In both cases, altered function or numbers of DCs resulted in lower systemic levels of IL-12 and increased susceptibility to T. gondii. In these models, resistance can be restored by treatment with IL-12, suggesting that DCs are an essential source of IL-12 during toxoplasmosis. Other studies have aimed to define which subsets of DCs are the most relevant sources of IL-12. Following the in vivo administration of soluble T. gondii antigens, the CD8α+ subset of DCs produces IL-12. More recently, mice lacking the transcription factor Batf3, which have a deficiency in CD8α+ DCs, have been shown to succumb to T. gondii associated with a severe IL-12 defect, reduced CD8+ T cell responses, and high parasite burdens. The finding that exogenous IL-12 restores survival of Batf3 KO mice is consistent with a model in which CD8α+ DCs are an essential source of IL-12.

Neutrophils are another source of IL-12 during toxoplasmosis, as they contain pre-stored IL-12 and can secrete this cytokine in vitro and in vivo in response to T. gondii. Additionally, there are reports that neutrophil depletion results in decreased levels of IL-12 and increased parasite replication. These findings are complicated by the realization that the strategy used to deplete neutrophils also affects other cell types, including inflammatory monocytes. Regardless, mice deficient in the chemokine receptor CXCR2, which is essential for neutrophil recruitment to the site of infection, have higher parasite levels in the CNS, suggesting a role for neutrophils during toxoplasmosis. Neutrophils are also implicated in other effector mechanisms that directly kill parasites, including phagocytosis, the release of reactive chemical species, and the formation of extracellular traps. While phagocytosis of T. gondii by neutrophils has been

observed in vitro, several groups have reported that p47phox, an enzyme component necessary for the oxidative burst generated by neutrophils following phagocytosis, is unnecessary for resistance to T. gondii. Indeed, in vivo imaging studies have observed swarms of neutrophils that congregate around infected cells, but the parasites present in the neutrophils appear to be largely intact. However, infection with T. gondii does induce increased extracellular DNA at the site of infection, which is dependent upon the presence of neutrophils, and this may be explained by the release of DNA from neutrophils to form extracellular traps. In vitro studies suggest that the formation of these traps results in decreased parasite vitality and may contribute to the control of T. gondii in vivo.

Monocytes are also required for resistance during toxoplasmosis, as mice deficient in the chemokine receptor CCR2, which in necessary for monocyte recruitment to the site of infection, exhibit increased susceptibility when challenged. Inflammatory monocytes produce IL-12 in vitro and in vivo when stimulated with T. gondii, however it is not clear whether they are a critical source of this cytokine. It has also been proposed that these populations contribute to the direct control of T. gondii through the generation of nitric oxide (NO), which inhibits parasite replication. In support of this model, inflammatory monocytes express inducible nitric oxide synthase (iNOS), the enzyme responsible for catalyzing the production of NO, and inflammatory monocytes are able to kill and inhibit the replication of T. gondii in vitro. Additionally, CCR2 KO mice given a low dose oral challenge of T. gondii succumb approximately 3–4 weeks after infection, and this is associated with decreased expression of iNOS and increased parasite burdens in the CNS. Although monocytes are clearly critical for survival during toxoplasmosis, their role is not limited to production of nitric oxide, as iNOS- deficient mice survive acute challenge, while deficiencies in monocyte recruitment can lead to acute susceptibility. Monocytes also produce IL-1 in response to soluble toxoplasma antigens, and this factor can enhance anti-toxoplasmic effector mechanisms in macrophages and astrocytes in vitro. Moreover, IL-1 can synergize with IL-12 to promote production of IFN-γ from innate and adaptive sources.

Natural killer (NK) cells are another innate population involved in immunity to T. gondii, and in mice that lack T cells they provide a limited mechanism of resistance through their ability to produce IFN-γ. NK cell activity peaks early during infection, and although their activity is elevated during chronic toxoplasmosis, they do not appear to be significant contributors to immunity during the chronic stage of infection. Consequently, most studies have focused on the early events that control NK cell activity, leading to a model in which IL-12 produced by other innate cells (e.g. neutrophils, monocytes and DCs) promotes NK cell-mediated production of IFN-γ . Human and murine NK cells can also be cytotoxic for cells infected with T. gondii, but it has been proposed that NK cells

become infected with the parasite following the lysis of infected cells, which may promote the dissemination of the parasite.

NK cells can also act to promote adaptive immune responses. Thus, in the absence of CD4+ T cells, they can provide help to the CD8+ T cell response. One mechanism by which this help is accomplished is by increasing IL-12 production from DCs through interactions dependent on the molecule NKG2D. Additionally, production of IFN-γ by NK cells has been implicated in the development of optimal CD4+ T cell responses.

Adaptive Immune Responses to T. gondii:

The importance of adaptive immune responses for resistance to T. gondii during human infection is demonstrated by the increased susceptibility of patients with primary or acquired defects in T cell function, and mice with deficiencies in B cells, CD4+ T cells or CD8+ T cells survive the acute stage of infection, but ultimately show increased susceptibility to T. gondii. Understanding how these different cell populations are integrated to provide long-term resistance has been challenging, but several advances in technology have improved our ability to study adaptive immune responses to T. gondii. For example, the identification of the molecular epitopes of T. gondii recognized by CD8+ T cells has allowed the measurement of antigen-specific CD8+ T cell responses during infection, and provided insight into the mechanisms by which antigen is presented. This has been complimented by the development of parasites that express model antigens such as ovalbumin, β-galactosidase, and EαRFP, as well as the use of two-photon imaging to allow visualization of immune cells following infection. These advances are currently allowing a higher resolution analysis of the events that mediate the control of T. gondii, and may also provide insight into the strategies used by this parasite to persist despite the presence of an array of anti-microbial effector mechanisms.

<u>CD4+ T cell responses: Initiation and mechanisms of controlling infection:</u>

As mentioned earlier, CD4+ T cells are critical for resistance during toxoplasmosis, as the emergence of severe toxoplasmosis is concomitant with the decline in T cell numbers in patients infected with HIV, and in mouse models, the lack of CD4+ T cells is associated with increased susceptibility during the chronic stage of infection. CD4+ T cells provide several critical regulatory functions in mediating resistance to toxoplasmosis. During the early stages of infection they contribute to optimal B and CD8+ T cell responses, and the ability of these cells to control chronic infection may be attributed to their production of cytokines such as IFN-γ, or their expression of CD40L (also referred to as

CD154), which can activate effector mechanisms in macrophages and other innate cells expressing CD40 on their surface.

The initiation of T cell responses requires that naïve CD4+ or CD8+ T cells encounter antigen presenting cells bearing their cognate antigen in the context of MHCII or MHCI molecules respectively, in conjunction with co-stimulatory and cytokine signals needed for T cell activation. During toxoplasmosis, ligation of the molecules CD28 and ICOS, expressed on the surface of T cells, contributes to the co-stimulatory signals, while IL-12 provides the cytokine signal required to promote proliferation and differentiation into populations that produce IFN-γ.

B cells, macrophages, and DCs are all capable of presenting antigen to CD4+ T cells, though DCs are generally considered the most crucial antigen presenting cell population in vivo. Following infection with T. gondii, multiple populations of DCs undergo expansion and acquire an activated phenotype. Additionally, challenge of mice with parasites engineered to express the model antigen EαRFP revealed that CD8α+ and plasmacytoid DCs (pDCs) express complexes of MHC class II and Eα, a peptide derived from EαRFP, on their surfaces. While these studies implicate pDCs and CD8α+ DCs as responsible for presenting antigen to CD4+ T cells during toxoplasmosis, the use of mice with deficiencies in specific DC compartments, as well as mouse models that allow for the selective depletion of DCs or DC subsets, may be useful to further define the roles of these populations in antigen presentation.

The mechanisms by which professional antigen presenting cells acquire parasite antigens for presentation in the context of MHCII are unclear, and there are several possible models to explain how this may be accomplished. Since there are multiple reports that murine DCs and monocytes infected with T. gondii express low levels of MHCII and co-stimulatory molecules, it has been suggested that infected cells would be poor presenters of antigen. Thus, antigen acquisition might occur through the phagocytosis of parasites, infected cells, or parasitic debris, or through the endocytosis of antigens secreted by the parasite. In vitro studies have demonstrated that murine DCs are able to present antigen derived from live and heat-killed parasites to CD4+ T cells. Because heat-killed parasites cannot invade cells, these data are consistent with a model in which antigen is acquired via the phagocytosis of parasites.

<u>Humoral immunity is essential for resistance to toxoplasmosis:</u>

It has long been recognized that infection with T. gondii promotes antibody responses, and that these antibodies can kill the parasite. Indeed, parasite-specific IgM, IgA, IgE and IgG2 antibodies have been isolated from human patients, and detection of parasite specific antibodies is an effective diagnostic tool to distinguish newly infected individuals from those in the chronic stage of infection. The critical role of antibody in immunity to T. gondii is demonstrated by the phenotype of μMT mice, which are deficient in B cells. These mice

develop apparently normal IFN-γ responses, but succumb to infection within 3–4 weeks following challenge, associated with high parasite burdens in the CNS. This increase in susceptibility is likely due to a lack of antibodies, as the passive transfer of antibodies confers protection to B cell-deficient mice. Antibodies can mediate their protective effects through a variety of mechanisms. In vitro studies have found that they can opsonize parasites for phagocytosis, block invasion, and also activate the classical complement pathway. The in vivo relevance of complement activation is illustrated by studies in which treatment of mice with an antibody that binds to the complement protein C3 results in acute susceptibility to toxoplasmosis. Additional studies are required to define the contribution of other antibody-mediated functions.

As mentioned previously, CD4+ T cells are necessary to promote optimal B cell responses and mice deficient in or depleted of CD4+ T cells display lower parasite-specific antibody titers. Furthermore, the increased susceptibility of CD4+ T cell-deficient mice can be ameliorated by the passive transfer of antibodies, indicating that the defect in antibody responses likely contributes to the failure to control parasite numbers. Curiously, infection with T. gondii results in severe disruption of splenic architecture and the loss of distinct B cell zones. Since B cell zones are considered the main location where CD4+ T cells provide help to B cells, this raises the question of whether there is a specialized microenvironment where T-B interactions occur when B cell zones are absent. Since disruption of secondary lymphoid structures is characteristic of many infections, murine models of toxoplasmosis may prove a useful system to interrogate the mechanisms by which CD4+ T cells help B cell responses, and the extent to which splenic architecture contributes to such interactions.

CD8+ T cell response: Initiation and control of parasite burden

Given that T. gondii is an intracellular pathogen, it is not surprising that CD8+ T cells, which are specialized to recognize and destroy cells infected with viral, bacterial and parasitic organisms, also have a critical role in mediating resistance to this infection. CD8+ T cells can control infection through the production of inflammatory cytokines such as IFN-γ, through CD40/CD40L interactions, and through the perforin-mediated cytolysis of infected host cells. Indeed, mice deficient in CD8+ T cells show increased susceptibility to toxoplasmosis, succumbing approximately 50 days post-infection. Furthermore, the adoptive transfer of CD8+ T cells from chronically infected mice, or mice vaccinated with an attenuated strain of T. gondii, is sufficient to confer resistance. Additional evidence comes from in vivo depletion studies using chronically infected mice, in which depletion of CD8+ T cells and CD4+ T cells results in reactivation of the infection and severe disease, but depletion of CD4+ T cells alone had limited impact.

As previously described, CD8+ T cell responses are initiated when naïve CD8+ T cells encounter their cognate antigen in the context of MHCI on the surface of antigen presenting cells, accompanied by co-stimulatory and cytokine signals. Some of the earliest studies on the CD8+ T cell response identified the Surface Antigen 1 (SAG-1) protein as a target of CD8+ T cells, although the specific peptide sequence of SAG-1 that the CD8+ T cells recognize remains unknown. More recently, technical advancements have accelerated the discovery of epitopes of T. gondii that are recognized by CD8+ T cells. Thus, in 2008, two studies identified peptides derived from T. gondii that are presented in the context of the H2-Ld allele of MHCI. These include peptides from the dense granule proteins GRA4 and GRA6, and the rhoptry protein ROP7. Of these, the GRA4 and ROP7 epitopes are conserved across multiple strains of T. gondii, whereas expression of the GRA6 epitope is limited to Type II strains. Another epitope, derived from the protein Tgd_057, is presented in the context of the MHCI allele H2-Kb, and is also conserved among multiple genotypic strains. The function of Tgd_057 is unclear, but despite the presence of a secretory signal, it localizes primarily to the cytosol of the parasite. It is of interest that all of these proteins, with the possible exception of Tgd_057, are secreted from the parasite.

While the cellular pathways by which phagocytosed antigens can come to be presented in the context of MHCI have been widely studied in a variety of systems, it is less clear how a cell infected with T. gondii can acquire antigen to be presented, given that the parasite resides in a specialized non-fusogenic vacuole. Several studies using reporter systems in which host cells respond to antigens derived from T. gondii have demonstrated that secreted antigens can enter the cytoplasm of infected cells. These antigens would then be transported from the cytosol into the endoplasmic reticulum by the Transporter Associated with Antigen Processing (TAP). This model is consistent with studies demonstrating that secreted antigens from T. gondii are preferentially presented to T cells. Alternatively, the PV can fuse with the endoplasmic reticulum, providing another mechanism by which antigens may escape sequestration and enter the protein transport pathway.

CD8+ T cell responses to T. gondii are influenced by help provided by CD4+ T cells. Although depletion of CD4+ T cells does not affect the magnitude of the CD8+ T cell response during the early stage of CD8+ T cell expansion and activation, CD4+ T cells are necessary for the maintenance of CD8+ T cell effector functions during the chronic stage of infection, and this help must be provided during the acute stage of infection. Further insights regarding the nature of CD4+ T cell help have been gained from studies using the attenuated vaccine strains of T. gondii ts-4 and cpsII, both of which require CD4+ T cell help for optimal protective CD8+ T cell responses. In current models, ts-4 vaccination stimulates CD4+ T cells to produce the growth factor IL-2, which provides an essential signal for CD8+ T cells. Indeed, neutralization of IL-2 results in

diminished CD8+ T cell responses and decreased protection. Other potential mechanisms by which CD4+ T cells may provide help include the licensing of DCs, or direct interactions with CD8+ T cells through CD40/CD40L interactions.

More subtle changes in CD8+ T cell responses may also help to explain differences in susceptibility among mouse strains. Whereas the C57B/6 inbred mouse strain succumbs to T. gondii during the chronic stage of infection, BALB/c mice are relatively resistant to toxoplasmic encephalitis. This difference in susceptibility has been genetically mapped to the MHC Class I H2-Ld allele, implicating CD8+ T cells as being responsible for this difference in susceptibility. The recent identification of an immunodominant epitope from the protein GRA6, recognized by CD8+ cells, that binds to the H2-Ld Allele has led to the hypothesis that recognition of this peptide is crucial for controlling T. gondii infection in BALB/c mice, and may account for the differences in virulence among mouse strains. Because expression of this epitope is restricted to Type II strains of T. gondii, its relative significance could be tested by replacing the peptide with the sequence present in Type I or Type III strains. Alternatively, it may be possible to tolerate mice to this epitope through vaccination, as has been reported in other systems. Regardless, these studies highlight the importance of GRA6 as a target for protective CD8+ T cells.

Effector mechanisms controlling T. gondii infection:

Cellular immunity mediates protection through the production of inflammatory cytokines such as IFN-γ. Other molecular signals, such as the cytokine tumor necrosis factor alpha (TNF-α) and CD40 ligation are also required for resistance during chronic toxoplasmos. This section describes how these distinct pathways are integrated to engage specific effector mechanisms required to directly control infection with T. gondii.

Nitric oxide inhibits replication of T. gondii:

Since the early 1980's, it was recognized that IFN-γ can activate macrophages to kill a variety of intracellular organisms, including T. gondii, and during the late 1980's it was reported that IFN-γ is also essential in vivo for resistance to T. gondii. These findings raised the fundamental question of how this cytokine promotes control of T. gondii and other pathogens. It was proposed that the protective effects of IFN-γ may be mediated by inducing increased synthesis of Nitric Oxide (NO). Consistent with this hypothesis, expression of inducible nitric oxide synthase (iNOS), the enzyme responsible for catalyzing the reaction that results in production of NO, is increased in macrophages by stimulation with IFN-γ, and NO inhibits replication of T. gondii in macrophages and other cell types. Importantly, IFN-γ alone is not typically sufficient to activate macrophages to kill T. gondii, and additional signals provided by factors like TNF-α or CD40L are required for optimal iNOS expression. In vivo evidence for a role of NO in controlling toxoplasmosis came from a study in which administration of the iNOS inhibitor aminoguanidine to infected mice resulted in increased parasite burdens. Subsequently, iNOS-deficient mice were developed and found to display increased susceptibility to toxoplasmosis, succumbing to disease in the chronic stage of infection. Although the specific mechanism by which NO inhibits replication of T. gondii remains to be determined, studies using intracellular bacterial pathogens

have shown that NO can inhibit bacterial enzymatic activity and directly damage DNA, which would preferentially affect pathogen replication and account for the static effects of NO.

IFN-γ mediates protection through the p47 GTPases:

The increased susceptibility of iNOS-deficient mice to toxoplasmic encephalitis clearly implicated iNOS in immunity to T. gondii, but also pointed toward iNOS-independent mechanisms by which IFN-γ mediates protection during the acute phase of infection. Like iNOS, members of the p47 GTPase family (also referred to as the immune related GTPase family (IRGs)) are also upregulated in response to IFN-γ, but the importance of this family was first apparent when mice that lack the p47 GTPase IGTP (Irgm3) were infected with T. gondii. These mice have normal IFN-γ responses, but succumb to acute toxoplasmosis due to high parasite burdens. Subsequent studies revealed other members of this family, including LRG-47 (Irgm1), IRG-47 (Irgd), IIGP1 (Irga6), and TGTP (Irgb6) to be involved in immunity to T. gondii as well. The specific mechanisms by which individual members of the p47 GTPase family promote the clearance of T. gondii are the subject of ongoing studies in many laboratories. There are reports that in IFN-γ activated cells p47 GTPases co-localize to the PV, which then develops a tight fitting morphology followed by a rough and disrupted appearance before being stripped away. Once free in the cytosol, the parasite egresses the infected cell or becomes permeabilized and killed.. In the latter studies the host cell was observed to undergo necrosis after killing the parasite. Additionally, other studies have observed the exposed cytosolic parasite to be disposed of by xenophagy, the process by which foreign bodies within a cell are eliminated using the same cellular machinery involved in autophagy. In further support of a role for autophagic machinery in immunity to T. gondii, the autophagy protein Atg5 has been found to be necessary for the disruption of the PV and resistance to this infection in vivo. Additionally, CD40 ligation has been observed to induce xenophagic elimination of parasites independently of p47 GTPases.

Given the important role of the p47 GTPases in immunity to T. gondii, it is not surprising that the parasite has evolved strategies to interfere with their function. At least three members of the p47 GTPase family, Irga6, Irgb6 and Irgb10, are phosphorylated by ROP18, resulting in changes in their functionality or cellular localization associated with increased virulence. Additionally, the recruitment of GBP1, a member of the guanylate-binding protein family (GBPs), to the PV is also inhibited by the parasite-derived virulence factors GRA15, ROP16 and ROP18. As the GBP family has recently been implicated in immunity to intracellular bacteria, this finding may be indicative of a role for GBPs in immunity to T. gondii, although further research will be necessary to directly test this hypothesis.

The role of tryptophan degradation as a defense mechanism:

IFN-γ can also mediate protective effects against T. gondii by promoting tryptophan degradation in a variety of infected cell types, including fibroblasts, macrophages, and brain cells. Treatment

of cells with IFN-γ results in the upregulation of the genes indolamine 2,3-dioxygenase 1 and 2 (IDO-1 and IDO-2), which catalyze the degradation of tryptophan. Because T. gondii is a natural tryptophan auxotroph, the increased degradation of tryptophan by host cells inhibits parasite growth. The in vivo relevance of this pathway is illustrated by the finding that long-term treatment of infected mice with inhibitors of IDO-1 and 2 results in increased susceptibility and increased parasite burdens during chronic infection. Interpretation of this finding is complicated by the fact that IDO has other known immune functions such as suppression of DC and effector T cell functions, as well as promotion of regulatory T cell responses.

Members of the TNF family are necessary for immunity to T. gondii:

In addition to IFN-γ, members of the TNF family such as CD40L, TNF-α and LT-α, are also required for protection during the chronic stage of infection. The critical role of TNF-α is demonstrated by studies in which neutralization of this cytokine results in increased susceptibility and higher parasite burdens. Additionally, mice deficient in TNF-α (TNF-α KO) or the components of its receptor (TNFR KO) succumb to infection approximately 3–4 weeks post-challenge despite having functional IFN-γ responses. TNF-α is produced by a number of cell populations in response to T. gondii or T. gondii antigens, including neutrophils, DCs, macrophages, microglia, and T cells. TNF-α synergizes with IFN-γ to promote anti-parasitic mechanisms in macrophages, as well as non-hematopoietic cells. In vitro studies have demonstrated that this can be mediated through the production of nitric oxide. Additionally, TNF-α KO mice, TNFR KO mice, and mice treated with a neutralizing antibody for TNF-α display decreased iNOS expression. Collectively, these data support a model in which TNF-α mediates its protection by inducing expression of iNOS. However, there are also data that suggest that susceptible TNFR KO mice infected with T. gondii can have appropriate levels of iNOS, suggesting that TNF-α can mediate protection through iNOS-independent mechanisms. Because TNF-α KO and TNFR KO mice are capable of surviving the acute stage of infection, it is clear that TNF-α is not required for the IGTP-mediated elimination of the parasite. This notion is also supported by in vitro studies, in which macrophages show no defect in their ability to kill parasites in the absence of TNF-α signaling. However, interpretation of these results is complicated by the finding that TNF-α plays a more prominent role in activating macrophages when concentrations of IFN-γ are limiting. Thus, the chronic susceptibility of mice deficient in TNF-α signaling may result from changes in the expression of IFN-γ during the course of infection rather than a deficiency in any one specific effector mechanism that is absolutely dependent upon TNF-α.

Another component of the TNF family involved in immunity to T. gondii is CD40L, which is expressed on T cells and binds to CD40 expressed on macrophages and other cell populations. The importance of CD40/CD40L interactions to promote immunity to T. gondii is evidenced by the increased susceptibility of patients with Hyper-IgM syndrome, a disease characterized by defective CD40L expression. During human toxoplasmosis, CD40/CD40L interactions are necessary to promote optimal production of IFN-γ and class switched antibody. In contrast, these interactions are not critical for production of IFN-γ in the murine model, yet mice deficient in CD40L display increased susceptibility during chronic infection. While CD40L can act

synergistically with IFN-γ to inhibit parasite replication, there is also evidence that CD40L can act independently of IFN-γ. One IFN-γ independent mechanism by which CD40L controls infection is through the induction of xenophagic killing of the parasite, which has been shown to be independent of the p47 GTPase family, but dependent upon the autophagic molecule Beclin-1. Beclin-1-heterozygous mice also demonstrate increased susceptibility to T. gondii infection, indicating that CD40-mediated xenophagy may be a unique and critical mechanism for controlling chronic toxoplasmosis.

Lymphotoxin alpha (LT-α) is another member of the TNF family essential for immunity to T. gondii. Like TNF and TNFR KO mice, LT-α KO mice succumb to this infection within the first 4 weeks, associated with a high parasite burden. These mice display functional but delayed IFN-γ responses and antibody titers, and decreased expression of iNOS. These defects may conceivably result from a critical role for LT-α in signaling to directly promote effector functions, or they may be a secondary consequence of the defective splenic architecture observed in LT-α KO mice.

Thus, cytokines and the effector mechanisms they induce are able to control toxoplasmosis, allowing the parasite and the host to co-exist. Parasite virulence factors or immunodeficiency can disrupt this equilibrium, leading to severe disease or the death of the host. [24]

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