

## Review

## Long-Term Consequences of Foodborne Toxoplasmosis: Effects on the Unborn, the Immunocompromised, the Elderly, and the Immunocompetent<sup>†</sup>

### ABSTRACT

In immunointact individuals, infection by the ubiquitous protozoan parasite *Toxoplasma gondii* is common, but clinical disease is rare; however, fetal and immunocompromised populations are at risk for clinical toxoplasmosis. *T. gondii* organisms persist as quiescent tissue cysts in various tissues of the body with the possibility of tissue cysts reactivating to actively multiplying parasites if there is a decline in the infected individual's immune system. In more recent years, there has been an increase in toxoplasmosis due to a steadily increasing immunocompromised population. *T. gondii* infections are controlled principally by the cellular immune system. Thus, individuals with defective cell-mediated immunity cannot control a *T. gondii* infection and if they have been infected previously, reactivation of a previous infection may occur. Congenital toxoplasmosis can cause severe complications in the fetuses of women who are infected with *T. gondii* during pregnancy. Toxoplasmosis can be serious in individuals with malignancies or AIDS. Since transplant recipients are immunosuppressed by drug treatment, they too are at risk for toxoplasmosis if they receive an organ from an infected donor. Vaccines against *T. gondii* suitable for human use have not been developed. No drug is available that can eliminate the encysted stage of the parasite; thus, infected individuals are always at risk for reactivation of the parasite if there is a failure of their immune system. More emphasis should be placed on the elimination of *T. gondii* by development of drugs which can eliminate the cyst stage in tissues and on development of vaccines suitable for protecting humans against infection or reactivation.

Key words: Long-term effect, *Toxoplasma gondii*, toxoplasmosis, immunocompromised, pregnancy, congenital toxoplasmosis

*Toxoplasma gondii* is a ubiquitous protozoan parasite capable of infecting almost all warm-blooded mammals and

birds; however, there is great variation in the extent of disease effects depending on the species infected. Fatal toxoplasmosis is seen in marsupials and New World monkeys, whereas chickens, swine, cattle, and horses seldom show clinical disease (43). While *T. gondii* infections are a major cause of abortion in sheep and goats, ewes generally have subclinical infections but goats may have severe clinical toxoplasmosis (42).

In immunocompetent humans, *T. gondii* infection is common but clinical toxoplasmosis is rare. It has been estimated that approximately 30% of adults in the United States and United Kingdom have antibodies against *T. gondii*, whereas in continental Europe 50 to 80% of adults are seropositive. Most infections are asymptomatic in immunocompetent persons. When symptoms are present, they are usually mild and self-limiting and individuals seldom seek medical attention (43, 51). Toxoplasmosis characteristically develops in *T. gondii*-infected individuals with undeveloped or impaired immune systems such as the developing fetus, the elderly, medically immunosuppressed patients, and patients with immunocompromising diseases. The fetus is at risk for toxoplasmosis if the woman is infected during pregnancy. There is a steadily increasing segment of the population whose immune status is compromised and who therefore are at risk for any opportunistic disease such as toxoplasmosis (43, 51). Recrudescence (reactivation of a previous infection with *T. gondii*), rather than a new infection, is the usual cause of toxoplasmosis in an immunocompromised patient (51). The long-term neurological and physiological consequences of toxoplasmosis to fetal, immunocompetent, and immunocompromised populations are discussed in this review.

### LIFE CYCLE OF *T. gondii*

*T. gondii* is an obligate intracellular parasite with a sexual life cycle which takes place only in the intestinal tract of cats (Felidae). The parasite has three basic stages: (i)

tachyzoites, proliferating forms which can invade a number of cell types; (ii) bradyzoites, slowly multiplying forms found encysted in various tissues (tissue cysts); and (iii) oocysts, environmentally resistant forms found only in feces of felines (43, 51, 70). All three forms are infectious.

The asexual cycle lacks host or cellular specificity and consists of the tachyzoite and bradyzoite forms. The tachyzoites (generated from ingested oocysts or bradyzoites) penetrate the host intestinal cells and multiply with ultimate destruction of the host cells and liberation of new tachyzoites. The newly released parasites attack other cells and eventually the organisms invade the mesenteric lymph nodes and the circulatory system. The resultant parasitemia allows the tachyzoites to spread to various bodily sites. As immunity to the parasite develops, the tachyzoites are transformed into the slowly multiplying bradyzoites, which accumulate in tissue cysts; each cyst may contain several hundred bradyzoites. The encysted bradyzoites are resistant to immune attack and cysts may persist in tissues for the life of the individual. Thus, a tissue cyst represents a quiescent stage in the life cycle of the parasite which, if the immune system breaks down, can release bradyzoites and provoke toxoplasmosis. Cysts may be found in heart, brain, and skeletal muscle as well as in other tissues (43, 51).

When the nonimmune cat ingests meat containing tissue cysts, intestinal enzymes digest the tissue cyst wall with release of bradyzoites. The bradyzoites penetrate the lamina propria of the intestine and multiply as tachyzoites. The parasites may then disseminate to extraintestinal tissues with eventual formation of tissue cysts; or bradyzoites invade the epithelial cells of the feline small intestine and form schizonts, which eventually give rise to male and female gamonts. After fertilization, a wall is formed around the fertilized female gamonts, which are excreted as unsporulated oocysts in cat feces. Oocysts sporulate and become infectious after a few days' exposure to the external environment (43, 51).

Infection can occur in humans and animals if they ingest sporulated oocysts present in soil or food that has been contaminated with cat feces. However, eating undercooked or raw meats containing tissue cysts appears to be the most common source of *T. gondii* for carnivores and humans (43). Smith (185, 186, 187) has discussed various aspects of foodborne toxoplasmosis.

### IMMUNOLOGY OF *T. gondii* INFECTION

Resistance against *T. gondii* is considered to be mainly cell mediated (92). Montoya et al. (126) have shown that human CD4<sup>+</sup> and CD8<sup>+</sup> T cells are cytotoxic to host *T. gondii*-infected cells in in vitro experiments. Transfer of lymphocytes from a *T. gondii*-seropositive animal to an uninfected animal confers resistance against a challenge infection of the parasite; passive transfer of *Toxoplasma* antibodies is not protective (83). However, Pavia (142) did find partial protection against the dissemination of the parasite in guinea pig recipients of immune serum. Binding of anti-*T. gondii* antibodies to the parasite surface leads to activation of complement and parasite lysis or to phagocytosis

by macrophages with the resultant death of the parasite (85). Antibody protection is limited, because *T. gondii* is an intracellular parasite and as such is protected from antibody action. Since both humoral and cellular immunity generally act in concert, it is probable that both types of immunity are necessary for resistance to *T. gondii* infection (142).

Upon primary infection by *T. gondii*, there is induction of a T helper cell type 1 (T<sub>H</sub>1) cytokine response (61, 95, 171, 205). The subsets T<sub>H</sub>1 and T<sub>H</sub>2 of CD4<sup>+</sup> T cells are defined by their cytokine profiles and immune functions. The T<sub>H</sub>1 subset produces the cytokines, interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumor necrosis factor (TNF). The T<sub>H</sub>1 cytokines activate macrophages, stimulate proliferation of cytolytic CD8<sup>+</sup> T cells, and give limited help to B cells (11, 81, 141). Thus, the products of the T<sub>H</sub>1 subset induce enhanced cell-mediated immunity leading to the identification of and elimination of host cells infected with pathogens (i.e., intracellular pathogens). The other subset, T<sub>H</sub>2, produces the cytokines, IL-4, -5, -6 and -10, which help B cells develop into antibody-producing cells (11, 81, 141). T<sub>H</sub>2 responses are appropriate in infections by extracellular organisms against which antibodies can act. The products of each T<sub>H</sub> subset have inhibitory actions against their counterparts: IFN-γ inhibits T<sub>H</sub>2 responses, whereas IL-4 and IL-10 inhibit T<sub>H</sub>1 responses (81, 139).

The basic immunological events that occur during infection with *T. gondii* can be described as follows: macrophages are activated by the parasite to produce IL-12, which is an obligatory cytokine in the induction of the T<sub>H</sub>1 response (203). IL-12 and TNF-α from activated macrophages stimulate IFN-γ production by natural killer (NK) cells, CD8<sup>+</sup> cells, and CD4<sup>+</sup> T cells. IFN-γ directs precursor T<sub>H</sub> cells toward a T<sub>H</sub>1 response (58, 59, 61, 94). In addition, IFN-γ inhibits the proliferation of the T<sub>H</sub>2 response (144). The cytokines produced by the T<sub>H</sub>1 subset, IFN-γ and IL-2, have been shown to play an important role in activating cell-mediated resistance to *T. gondii* (174, 198). A number of immune cells can identify and destroy host cells infected with *T. gondii*. These include lymphokine-activated killer cells (196), lymphokine-activated macrophages (60, 178), CD4<sup>+</sup> cytotoxic T lymphocytes (7, 32, 86, 216) and CD8<sup>+</sup> cytotoxic T lymphocytes (37, 86, 138, 199).

Following the host's initial T<sub>H</sub>1 response to *T. gondii* infection, there is suppression of T-cell lymphoproliferative responses and downregulation of IL-2 and IFN-γ production mediated by IL-10 and IL-4 (74, 95, 205). IL-4 is produced by T cells and basophil-mast cells and IL-10 is produced by T and B cells, mast cells, monocytes-macrophages and keratinocytes (81). Morris et al. (127) have shown that IL-12 is a strong inducer of IL-10 production and thereby limits its own effects by inducing the downregulating cytokine IL-10. IL-10 and IL-4 direct the immune system away from the production of T<sub>H</sub>1 cells toward the production of T<sub>H</sub>2 cells with shut-down of IFN-γ and IL-2 production and cell-mediated immunity (81, 95). Downregulation of T<sub>H</sub>1 response by IL-10 and IL-4 with induction of a T<sub>H</sub>2 response is a strategy which *T. gondii* employs to evade IFN-γ-dependent cell-mediated immune destruction (60). In *T. gondii* infections, the immune system

must come to an equilibrium leading to the production of immune-unresponsive encysted bradyzoites from tachyzoites or the host will succumb to overwhelming toxoplasmosis. *T. gondii*-specific CD4<sup>+</sup> T-cell clones isolated from healthy seropositive individuals belonged to the T<sub>H</sub>0 subset, which produced IL-2, IFN- $\gamma$ , IL-4, IL-5, and IL-10 (81, 148). The functions of the T<sub>H</sub>0 cells are largely unknown, but they may provide help for B-cell function, act as precursor cells for the T<sub>H</sub>1 and T<sub>H</sub>2 subsets, and probably have functions intermediate between those of T<sub>H</sub>1 and T<sub>H</sub>2 cells (47, 81, 194). Is it possible that the T<sub>H</sub>0 subset plays a role in tachyzoite transformation into encysted bradyzoites and helps in maintaining that state?

Using T cells stimulated by *Toxoplasma* antigens, Däubener et al. (35) established 46 different *Toxoplasma* antigen-specific human CD4<sup>+</sup> T-cell clones. The supernatants from 44 of the 46 clones were able to inhibit the growth of *T. gondii* in infected glioblastoma cells. The active principle was IFN- $\gamma$ , indicating that the T cells with toxoplasmosis activity were of the T<sub>H</sub>1 subtype (35). Two of the 46 T-cell clones did not induce toxoplasmosis. The supernatants from those T cells contained IL-4, thereby indicating that these cells were of the T<sub>H</sub>2 subtype. Thus, *T. gondii* antigens can induce both T<sub>H</sub>1 and T<sub>H</sub>2 T helper cells; however, most of the induced T cells were of the T<sub>H</sub>1 subtype (35). It would be of interest to determine which antigens induce a particular T cell subtype.

An immune response has generally been invoked to explain the in vivo formation of encysted bradyzoites. Using SCID mice deficient in both T and B cells, Gazzinelli et al. (59) established that *T. gondii* tissue cysts containing bradyzoites developed in the brains of SCID mice to which recombinant IL-12 was administered, whereas tachyzoites but no tissue cysts were found in control SCID mice. Since IL-12 is an important immunomodulating cytokine, this work suggests that immune functions are influential in the development and encystment of bradyzoites.

Conversion of bradyzoites to tachyzoites (reactivation) results from downregulation of IFN- $\gamma$  and TNF- $\alpha$  expression (57). Using murine bone marrow-derived macrophages infected with tachyzoites, Bohne et al. (13, 14) found that activation of the infected macrophages with IFN- $\gamma$  or bacterial lipopolysaccharide inhibited the replication of *T. gondii* tachyzoites and induced bradyzoite-specific antigens. Induction of bradyzoite antigens was closely correlated to activated macrophage production of nitric oxide, which reduced parasite replication. Use of sodium nitroprusside as a source of exogenous NO led to induction of bradyzoite antigens (14). NO may be acting as a molecular trigger of tachyzoite-to-bradyzoite conversion (14, 57). Thus, bradyzoite formation appears to be closely related to inhibition of tachyzoite replication by NO. Soëte et al. (190) demonstrated that tachyzoite growth was inhibited in infected Vero or human foreskin fibroblast cells treated with IFN- $\gamma$ . However, there was little bradyzoite-specific protein induction and there was no tissue cyst formation. The apparent conflict in the results obtained by Bohne et al. (13) and Soëte et al. (190) may be due to the different host cellular systems used.

Dardé et al. (34) have shown that bradyzoites were

formed in vitro in human fibroblast culture in the absence of immune responses. Foulet et al. (49) demonstrated the presence of *T. gondii* tissue cysts in the brains of genetically athymic nude rats deficient in T cells, thus indicating that encystment of the parasite is independent of cellular immune functions.

In tissue culture cells infected with *T. gondii* tachyzoites, bradyzoite-specific proteins were induced when the temperature was increased from 37 to 43°C or when the pH was increased to 8.0 (190). The upshift in temperature and pH also induced formation of tissue cysts with a structure similar to those seen in infected mouse brain. Although Soëte et al. (190) did not investigate the stress-induced bradyzoite-specific proteins, they suggested that the proteins might be stress proteins. Lyons and Johnson (111) demonstrated the presence of a parasite 70-kDa heat-shock protein (HSP70) in mice during infection by *T. gondii*. These authors concluded that HSP70 was induced by immunological stresses associated with mouse infection by *T. gondii*. Since Soëte et al. (190) showed tissue cyst formation under stress conditions, it may be possible that HSP70 induced by immunological stress leads to bradyzoite and tissue cyst formation. Adjustment of *T. gondii*-infected human fibroblasts to pH 6.8 or 8.2 increased tissue cyst formation (210). Alkaline stress conditions may be important in the conversion of tachyzoites to bradyzoites and in tissue cyst formation.

*T. gondii* tachyzoites are quite sensitive to antimitochondrial drugs: inhibition of tachyzoite mitochondrial activity stimulates the differentiation of the parasite to the bradyzoite stage (202). Thus, the trigger for tachyzoite transformation to the bradyzoite stage may depend on inhibiting the metabolic activities of tachyzoite mitochondria. Very little is known concerning the host and/or parasite origins of the signals for bradyzoite formation from tachyzoites with eventual tissue cyst formation (90). Tachyzoite-bradyzoite interconversion has been reviewed recently by Gross et al. (67).

An acute infection with *T. gondii* leads to nonspecific immunosuppression of both T and B cell activities (92). There may be suppression of antibody response to vaccination with antigens such as sheep red blood cells, poliovirus, and tetanus toxoid (20, 77, 197, 200). *T. gondii* infection with subsequent immunosuppression also led to increased allograft survival in mice that had received a skin graft from allogenic mice (77).

Concanavalin A is a selective T-cell mitogen, which stimulates T cells to undergo mitosis and proliferate (209). When concanavalin A was used to stimulate mouse T cells (from spleen), the proliferative response of cells from *T. gondii*-infected mice was significantly depressed when compared to stimulated uninfected spleen cells (20). The suppression of concanavalin A stimulation of spleen cell proliferation during the acute stage of infection correlated with the production of reactive nitrogen intermediates (RNI) by the spleen cells. Addition of an inhibitor of RNI production to spleen cultures from *T. gondii*-infected mice led to partial recovery of the proliferative response (20).

A *T. gondii* infection can exacerbate viral infections in sheep and lambs. Lambs, dually infected with *T. gondii* and louping-ill virus showed higher and longer persisting virus

titers than lambs infected with the virus alone (152). Dually-infected sheep showed a higher mortality than control sheep infected with virus alone. Sheep infected with only the parasite did not demonstrate mortality. The work of Reid et al. (152) indicates *T. gondii* infection in sheep and lambs can interfere with the immune response to louping-ill virus and probably to other viral and bacterial infections.

Interestingly, *T. gondii*-infected mice limit the proliferation of intravenously inoculated *Cryptococcus neoformans* yeast cells which colonize the brain; however, protection is limited to the brain since there is little or no protection against yeast colonization of the lungs with intratracheal instillation of the yeast (3). The phenomenon of parasite protection against brain colonization by the yeast is mediated by the inflammatory reaction against bradyzoites released by sporadic brain tissue cyst rupture. Rupture of *T. gondii* tissue cysts in the brain is intermittent and is followed by influx of inflammatory cells and macrophages which take up and destroy the debris and parasites associated with tissue cyst rupture (3, 46, 52). The protective phenomenon against *C. neoformans* is tissue cyst associated since infection with a non-tissue cyst-forming strain of *T. gondii* did not protect against yeast colonization of the brain even though that particular strain of the parasite induces a strong immunity (3). Thus, mice infected with *T. gondii* are protected against cerebral cryptococcosis but are not protected against pulmonary cryptococcosis.

### CONGENITAL TOXOPLASMOSIS

Women who are infected with *T. gondii* during pregnancy are generally asymptomatic; however, a few may present with enlarged cervical lymph glands. During the stage of tachyzoite dissemination (parasitemia), an infection of the placenta may be followed by infection of the fetus (28, 212). Even though their mothers are infected, approximately 60% of fetuses escape infection (43, 104, 153). The incidence of fetal infection depends on the stage of gestation when the woman is infected (Figure 1). Transmission of the parasite to the fetus is greater if the mother is infected late in pregnancy. If infection occurs early during gestation (first trimester), there is less chance for fetal infection (Figure 1). However, the risk of miscarriage, stillbirth, or severe disease in the newborn is much higher if the mother's infection is acquired early during pregnancy (28, 104, 212). In the United States, congenital toxoplasmosis has been estimated to range from 1 to 10 cases per 10,000 live births (212). Carter and Frank (21) have estimated the incidence of congenital toxoplasmosis in Canada at 4 to 40 cases per 10,000 live births. Worldwide, the incidence ranges from <10 to 30 cases per 10,000 live births (99).

Healthy immunocompetent women who are seropositive for *T. gondii* do not transmit the parasite to their fetuses (104, 153, 212). However, since there is a lag time before immunity to *T. gondii* develops, pregnancy should be delayed for at least 6 months after infection in order to ensure that the fetus will not acquire congenital toxoplasmosis (212). The seropositive pregnant woman with chronic toxoplasmosis (i.e., her tissues contain bradyzoites in cysts)

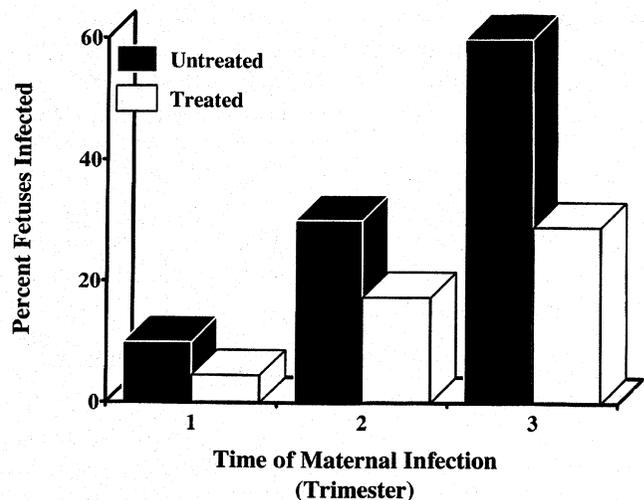


FIGURE 1. Percent *T. gondii* fetal infection as function of gestation stage when maternal infection occurred: role of treatment. As soon as maternal infection was diagnosed, the patient was treated with spiramycin for the remainder of pregnancy. If fetal infection was confirmed, spiramycin was alternated with pyrimethamine-sulfadiazine for the remainder of the pregnancy. Plotted from data in Forestier et al. (48) and Wong and Remington (212).

and who also has AIDS may, because of her immunologic deficiencies, have reactivation of the parasite. The resulting parasitemia can be life-threatening to the woman and there may be transmission of both *T. gondii* and HIV to the fetus (113, 153, 212). It is probable that the fetus of any *T. gondii*-seropositive immunosuppressed or immunocompromised pregnant woman is at risk for congenital toxoplasmosis. Indeed, D'Ercole et al. (38) reported congenital toxoplasmosis in several pregnancies in a woman immunocompromised with lupus erythematosus, splenectomy and corticosteroid therapy.

#### Long-term consequences of congenital toxoplasmosis

Four patterns of congenital toxoplasmosis in live newborns may be seen: (i) a neonatal disease with systemic signs; (ii) a disease occurring in the first months of life; (iii) sequelae recognized during late infancy, childhood, adolescence or adulthood; and (iv) relapses (29). Approximately 60% of neonates born alive to *T. gondii*-infected mothers are not infected. Of the 40% of live babies who contract infection in utero, about 26% have subclinical infections and appear normal at birth; about 10% show clinical signs of congenital toxoplasmosis (6% show mild symptoms and 4% are severely affected). Three to four percent die during the neonatal period (43). Only a minority of neonates infected in utero will have severe manifestations at birth or later in infancy. These symptoms include at least one of the components of the triad hydrocephalus, intracranial calcification, and chorioretinitis (71). Cook (28) added a fourth component: convulsions. Hydrocephalus is abnormal accumulation of spinal fluid in the brain; intracranial calcification is abnormal accumulation of calcium deposits in the brain; and chorioretinitis is inflammation of the outer membrane (choroid) and retina of the eye.

Congenital toxoplasmosis may involve the ocular sys-

tem, the auditory system, and the central nervous system. Other organs may be affected such as the heart, lungs, and liver (28, 65, 71, 153). Meenken et al. (123) observed endocrinological involvement in cases of severe congenital toxoplasmosis. The endocrinological sequelae were probably related to damage in the hypothalamic area of the brain associated with hydrocephaly. Lynfield and Eaton (110) have termed *T. gondii* a teratogen when it causes an infection in utero.

Approximately 85% of infants with congenital toxoplasmosis appear normal at birth (especially those born of mothers who were infected late in pregnancy); however, serious sequelae may develop several months or years later in these "normal" babies (104, 193, 211, 212). The remainder of the infants, particularly those from mothers infected early in pregnancy, will show clinical signs of toxoplasmosis at birth. It is tragic for parents who have a baby with visible signs of congenital toxoplasmosis, particularly if those signs involve the central nervous and ocular systems. It is doubly tragic for the parents of a baby who has diagnosed subclinical toxoplasmosis (or who is undiagnosed), and appears normal but develops symptoms of toxoplasmosis in late infancy, childhood, or adolescence. Thus, subclinical toxoplasmosis in the newborn is not an innocuous disease but may result in loss of vision and psychomotor and mental impairment later in life. About 85% of children born with untreated subclinical toxoplasmosis will eventually develop one or more bouts of chorioretinitis with the possibility of vision loss. Latent central nervous system infections may eventually result in intellectual impairment, seizures, behavior problems and/or deafness (104, 153, 212).

#### Treatment of congenital toxoplasmosis

Treatment of the pregnant woman as soon as *T. gondii* infection is diagnosed is beneficial to the fetus (Figure 1). The incidence of fetal infection was decreased by approximately half when the mother was treated with spiramycin. It is recommended that spiramycin be continued until the status of the fetus is finalized (amniocentesis, chorion villus sampling, analysis of fetal blood or ultrasound examination). If the fetus is not infected, spiramycin should be continued to the end of pregnancy, since the placenta remains infected for the duration of the pregnancy (192, 193, 212). If the fetus is infected, then the pregnant woman should be treated with a pyrimethamine-sulfadiazine-folinic acid combination alternating with spiramycin starting with the second trimester and continuing to the end of pregnancy (192, 193). Not only does treatment of the mother reduce the incidence of *T. gondii* transmission to the fetus; treatment of the fetus during pregnancy also decreases the severity of congenital toxoplasmosis. Infected babies born to treated mothers show increased subclinical toxoplasmosis and a decreased incidence in benign and severe toxoplasmosis (Figure 2). Daffos et al. (33) also concluded that prenatal therapy in cases of *T. gondii* maternal infection reduces the severity of the presentation of congenital toxoplasmosis.

Congenital toxoplasmosis of neonates, both clinical and subclinical, should be treated. Pyrimethamine-sulfadiazine-folinic acid alternately with spiramycin should be given during the first year of life (28, 29, 140, 153, 193). After one

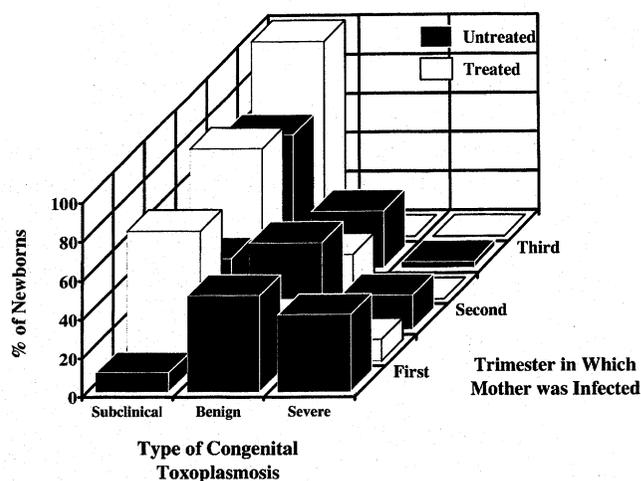


FIGURE 2. Severity of congenital toxoplasmosis in live newborns with and without in utero treatment. Treatment consisted of spiramycin as soon as the mother was diagnosed with *T. gondii* infection. When fetal infection was diagnosed, pyrimethamine-sulfadiazine-folinic acid alternated with spiramycin was given to the mother after 17th week of gestation. Treatment was continued until the end of pregnancy. Subclinical congenital toxoplasmosis was diagnosed in newborns with no visible symptoms. Neonates with benign toxoplasmosis were defined as having isolated symptomatic signs: intracerebral calcifications with normal neurological status and chorioretinitis scars without vision impairment. Severe toxoplasmosis included hydrocephaly or microcephaly with abnormal neurological status and bilateral chorioretinitis with vision impairment. Plotted from data in Hohlfeld et al. (79).

year of age, treatment should be given only if there is a relapse (reappearance of chorioretinitis or CNS [central nervous system] symptoms).

Studies of Guerina et al. (68), McAuley et al. (118) and Roizen et al. (164) indicate that treating infected neonates with a one-year regimen of pyrimethamine-sulfadiazine-folinic acid improved the pathological picture of congenital toxoplasmosis. The neurological and developmental outcomes were significantly better for treated infants than outcomes reported in the older literature for untreated patients. However, it is too soon to conclude definitely that the long-term outcome of treated children in these studies will be better than anticipated. In the work of Guerina et al. (68), McAuley et al. (118) and Roizen et al. (164), infants were treated after they were born; they had not been treated in utero. It is probable that if the mothers had received treatment during pregnancy, the disease outcomes would have been more promising.

Stray-Pedersen (193) recommended against therapeutic abortion based only on maternal *T. gondii* infection; if the woman is treated during pregnancy, abortion is needless in 80 to 90% of the cases. Wong and Remington (212) stated that therapeutic abortion should be a choice only if the fetus is definitely infected. With increased use of antenatal diagnosis, termination of pregnancy for toxoplasmosis is becoming rare. Abortion should be limited to cases where ultrasound abnormalities indicate that the fetus is suffering from severe cerebral toxoplasmosis (147).

Spiramycin, a macrolide antibiotic, is safe, virtually free

from side effects, and nonteratogenic (28, 122). While treatment of the mother with spiramycin reduces the frequency of placental transmission of the parasite to her fetus (Figure 1), once the fetus has been infected the antibiotic does not appear to modify the fetal infection process (28, 212). Therefore, when fetal infection has been confirmed, pyrimethamine-sulfadiazine-folinic acid should be added to the mother's treatment regimen. Pyrimethamine and sulfadiazine act synergistically against *T. gondii*. Pyrimethamine inhibits dihydrofolic acid reductase in the parasite whereas sulfonamides inhibit dihydrofolic acid synthetase. Both pyrimethamine and sulfadiazine depress bone marrow with resultant platelet decrease, leukopenia, and anemia (193). The administration of folinic acid counteracts bone marrow suppression (122). Folic acid cannot be used since it antagonizes the antitoxoplasma effect of pyrimethamine. It is believed that pyrimethamine is potentially teratogenic and should not be given during the first trimester (212) but this has been disputed by Cook (28) and Stray-Pedersen (193). Spiramycin, pyrimethamine, and sulfadiazine are effective against *T. gondii* tachyzoites, but at present no drug or combination of drugs available to clinicians are effective against bradyzoites (122). Thus, only acute toxoplasmosis with actively multiplying tachyzoites can be treated; the infected individual still has quiescent bradyzoites in the tissue cyst form which are a potential source of tachyzoites if the immune system breaks down.

#### *Vaccination as a means of controlling congenital toxoplasmosis*

Vaccination of young women before pregnancy would appear to be an ideal way to deal with the problem of congenital toxoplasmosis. However, at present, there is no vaccine available for use in humans. Indirect procedures to protect against *T. gondii* infections such as vaccination of cats do not appear to be feasible in view of the large number of feral cats that are present in the environment (8). A commercial vaccine is available that reduces abortion and neonatal mortality in sheep (19). The ovine vaccine contains live tachyzoites from strain S48 of *T. gondii*, which has lost the ability to develop bradyzoites and thus does not persist in sheep. The successful vaccination of sheep suggests that vaccination of humans as a means of controlling toxoplasmosis is possible.

A live vaccine probably is not suitable for humans, whereas recombinant antigens from the various developmental stages of *T. gondii* do have potential as human vaccines (8). Protection against *T. gondii* infection is primarily mediated by the cellular immune system (150). Therefore, recombinant antigens (encapsulated, combined with the proper adjuvant or microbial shuttle) to be used as anti-*T. gondii* vaccines must be able to induce *Toxoplasma*-specific cytotoxic T lymphocytes. In addition, the recombinant antigen should induce immune responses in the gut mucosa with production of secretory IgA and thereby prevent tachyzoite invasion of intestinal cells and mesenteric lymph nodes (8, 150, 173). It has been suggested that cell-mediated immunity can be increased by the simultaneous administration of antigen and interleukin-12 (72, 215). The formation

of IgA may be augmented by administration of IL-5 or transforming growth factor  $\beta$  (TGF- $\beta$ ) with vaccines (201).

Oral or intranasal dosing of mice with a cholera toxin-*T. gondii* tachyzoite antigen combination gave enhanced protection against a lethal challenge of tissue cysts (16, 17, 18, 36). The use of cholera toxin as an adjuvant induced increased anti-*T. gondii* cell-mediated immunity and secretory IgA response. Recent studies indicate that oral immunization with antigens combined with the B subunit of the heat-labile enterotoxin of *Escherichia coli* will induce mucosal antibodies (129).

By complexing antigens with proteosomes, Lowell et al. (103) and Orr et al. (135) produced vaccines that induced mucosal immunity in mice. Proteosomes are preparations of neisserial outer-membrane-protein vesicles (135). It is probable that complexing of proteosomes with tachyzoite antigens could induce mucosal immunity to prevent intestinal invasion by *T. gondii*.

Iscoms are immune-stimulating complexes composed of saponin Quil A, cholesterol, and phospholipids in which antigens are incorporated. Combining *T. gondii* antigens with iscoms led to induction of substantial humoral and cellular immune responses in mice and sheep (109). The T-cell response elicited by *T. gondii*-iscom complexes is predominantly the  $T_H1$  type with production of high levels of IFN- $\gamma$ . However, anti-*T. gondii* vaccines are in the experimental stages at the present time.

The oral or parenteral administration of the Schiff base-forming compound, tucaresol (4(2-formyl-3-hydroxyphenoxy)methyl)benzoid acid) during an infection may be a new approach to increasing the cellular immune response since tucaresol promotes a  $T_H1$  cytokine response in mice (159). It would seem feasible to test tucaresol in cases of *T. gondii* infections, since promotion of  $T_H1$  cell response would lead to destruction of the parasite.

#### *Role of hormones in congenital toxoplasmosis*

Female mice are more susceptible to *T. gondii* infection than male mice. Infected females had a higher mortality rate, and in the surviving female mice there were more intense inflammatory brain lesions and a greater number of brain tissue cysts than in males (162). The decreased survival and increased brain tissue cyst burden of female mice appear due to the failure of females to respond as quickly as males in terms of T-cell proliferation and IFN- $\gamma$  production (162). The differences between male and female responses to *T. gondii* are probably hormone related. Exposure to pharmacologic concentrations of estrogenic compounds (i.e., levels that are seen in pregnancy) such as 17 $\beta$ -estradiol, diethylstilbestrol, and  $\alpha$ -dienestrol increased the susceptibility of female mice to *T. gondii* infection as measured by brain tissue cyst formation (149). However, administration of weakly estrogenic compounds such as 5 $\alpha$ -dihydrotestosterone, progesterone, and zearalenol did not alter the female host resistance to infection by the parasite. Kittas and Henry (96) established that the estrogen hexoestrol greatly increased the number of brain tissue cysts in *T. gondii*-infected gonadectomized male or female mice. Luft and Remington (106) found that pregnant mice were more readily infected

by *T. gondii* than were virgin mice. The difference in ease of infection probably reflects hormonal differences. Thus, the fetus is at increased risk for *T. gondii* infection, since pregnancy and its associated hormones appear to increase the risk of parasite infection of females.

#### *Role of T<sub>H1</sub> and T<sub>H2</sub> cells in congenital toxoplasmosis*

It is interesting that there is localized T<sub>H2</sub>-type cytokine production at the maternal-fetal interface (102). Thus, during pregnancy, the maternal immune response is biased toward antibody production and away from cell-mediated immunity in the vicinity of the fetal-placental area. Cell-mediated immunity is downregulated to prevent immune rejection of the fetus (208). Lin et al. (102) demonstrated the presence of IL-4, -5 and -10 in cells derived from the fetal-placental area in mice. IFN- $\gamma$  was detected during the first trimester but not during the third. Localized downregulation of IFN- $\gamma$  and IL-2 is probably necessary, because these cytokines have been shown to increase the rate of fetal resorption in mice (24). None of the T<sub>H2</sub> cytokines were produced by cells from the spleen or mesenteric lymph nodes of pregnant mice (102). The absence of a T<sub>H1</sub> response at the fetal-placental interface suggests that the placenta may be readily infected by *T. gondii* during pregnancy with facile transfer of the parasites to the fetus.

A strong T<sub>H1</sub> response against intracellular pathogens may compromise pregnancy; however, pregnancy may compromise the protective T<sub>H1</sub> response against intracellular pathogens (128). Therefore, during pregnancy, latent infections due to intracellular pathogens such as cytomegalovirus, Epstein-Barr virus, *Mycobacterium tuberculosis* or *M. leprae* can reactivate to cause full-blown disease (207). Resistance to these intracellular pathogenic organisms is cell-mediated and the decrease in a T<sub>H1</sub> cytokine response during pregnancy allows reactivation of these latent infections to disease states. It would be expected that latent toxoplasmosis would reactivate during pregnancy; however, reactivation of *T. gondii* does not normally take place in seropositive immunocompetent pregnant women (104, 212). The reason for the lack of reactivation of the intracellular parasite *T. gondii* during pregnancy is not clear. Latent toxoplasmosis does have a well-defined tissue cyst stage and may not necessarily reactivate like other latent infections during pregnancy. Would the hormones produced by the pregnant female prevent reactivation of the *T. gondii* tissue cysts? This seems doubtful, because HIV-infected women who are seropositive for the parasite before pregnancy may reactivate and transmit both HIV and *T. gondii* to the fetus (212). The hormone status in HIV-infected pregnant women is probably similar to that of normal pregnant women, but the T-cell pattern does change. Gazzinelli et al. (62) has shown that there must be simultaneous depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells for reactivation of chronic toxoplasmosis in mice. However, such massive depletion of T cells would not occur during normal pregnancy.

#### *Animal models for human congenital toxoplasmosis*

When Sprague-Dawley rats (44), Fischer rats (217), and BALB/c or BALB/K mice (161) seronegative for *T. gondii*

were infected with various strains of the parasite during pregnancy, progeny with congenital toxoplasmosis were produced. Animals infected 8 to 10 weeks before pregnancy (and shown to be seropositive) did not produce infected progeny. Reinfection of seropositive mice or rats with *T. gondii* during pregnancy did not lead to congenital toxoplasmosis in their litters. Animals infected during pregnancy that gave birth to congenitally infected progeny did not produce infected litters in subsequent pregnancies. These animal models mimic human congenital toxoplasmosis and should be suitable models for vaccine and therapeutic studies. In addition, these models could provide information concerning the pathology of human congenital toxoplasmosis.

#### *Prevention of congenital toxoplasmosis*

The prevention of congenital toxoplasmosis demands a three-pronged attack (71, 212).

a. **Primary prevention** is aimed at preventing *T. gondii* infection during pregnancy through education of women prior to their first pregnancy. An educational program should include discussion and demonstration of food safety and other sanitation measures that can be used to prevent *T. gondii* infection. One study of the impact of education on the incidence of toxoplasmosis during pregnancy indicated that prevention instruction is of limited usefulness (50); however, another study indicated that an educational program aimed at reducing *T. gondii* infection during pregnancy would be effective in decreasing the incidence of infection (22). Another aspect of primary prevention would include immunization of all *T. gondii*-seronegative females of child-bearing age; however, vaccination to prevent infection by the parasite is not feasible at the present time.

b. **Secondary prevention** consists of serological screening of *T. gondii*-seronegative women during pregnancy. If the individual tests seropositive during pregnancy, then treatment with spiramycin should be initiated to lessen the chances of fetal infection. However, if screening of the fetus indicates infection, then the woman should be treated with both spiramycin and the pyrimethamine-sulfadiazine-folinic combination to decrease the severity of toxoplasmosis to the fetus. Treatment of *T. gondii*-pregnant women has been shown to be beneficial to infected fetuses (33, 192, 193, 212).

c. **Tertiary prevention** is aimed at alleviation of the consequences of congenital toxoplasmosis in newborns by continuation of anti-*T. gondii* treatment for at least one year. A number of studies (68, 118, 164) suggest that continued antibiotic treatment of infected newborns does decrease the severity of congenital toxoplasmosis.

In primary prevention by health education, it is necessary for the physician to repeat the message on how to prevent toxoplasmosis during pregnancy at every visit of the patient to the clinic (27). It is also necessary to repeat serological screening for toxoplasma antibodies in the pregnant woman at every visit to the physician until the baby is born and it is necessary to do repeated screening of the fetus once the mother is infected to determine if fetal transmission of the parasite has occurred (27).

In an editorial in the *New England Journal of Medicine*

in 1988, McCabe and Remington (119) made the observation that congenital toxoplasmosis is a preventable disease and that the time had come for the medical and public health systems to initiate steps to prevent it. For many years, France has had a program in place which is directed at preventing congenital toxoplasmosis. This program combines education, serological screening of both the pregnant woman and her fetus, treatment in utero if infection is detected and treatment of the baby after birth (145, 154). Such a comprehensive program is still not available in the United States and now would appear to be the time for the three-pronged attack on congenital toxoplasmosis to be introduced. Initiation of the three-pronged attack in the United States would decrease the incidence of the disease and make the course of toxoplasmosis in infected babies significantly milder.

#### *Economic losses due to congenital toxoplasmosis*

Roberts et al. (163) have estimated that the annual economic loss due to congenital toxoplasmosis in the United States ranges from \$0.4 to \$8.8 billion (1992 dollars). The wide range of the estimate is due to the uncertainty of the number of infected babies (420 to 10,920) born each year (163). The costs incurred through congenital toxoplasmosis include medical costs, income losses, and costs due to special education and/or special residential care. In the United Kingdom, the costs due to clinical cases of congenital toxoplasmosis (subclinical cases were excluded) were estimated at \$1.2 to \$12 million (1992 U.S. dollars). The estimated number of cases of congenital toxoplasmosis that occur annually in the United Kingdom range from 243 to 2,428 (163). Thus, the number of congenital toxoplasmosis cases is low but the costs are disproportionately high.

Lappalainen et al. (100), using decision and sensitivity analyses, concluded that serological screening and treatment for congenital toxoplasmosis in Finland would be beneficial in terms of cost and should be considered. When Roberts et al. (163) assumed that 4,179 cases of congenital toxoplasmosis occurred in the United States in 1992 (i.e., one case per 1,000 live births), the costs totaled \$5,256 million (1992 dollars). Therefore, the economic loss for each case of congenital toxoplasmosis is in excess of \$1 million. Each child born with congenital toxoplasmosis represents a significant loss to the economy of the United States. Even more importantly, congenital toxoplasmosis leads to a loss of human potential since many of the diseased children can never realize their full promise as members of the human community.

## **TOXOPLASMOSIS AND MALIGNANCY**

Toxoplasmosis is uncommon in cancer patients, but when it is seen, the patient usually has Hodgkin's disease (87, 107). However, toxoplasmosis has been described in patients with non-Hodgkin's lymphoma, lymphosarcoma, reticulum cell sarcoma, acute and chronic leukemias, multiple myeloma, myeloid metaplasia, hairy cell leukemia and angioimmunoblastic lymphadenopathy (87, 107). Reactivation of latent toxoplasmosis in cancer patients is probably

due to the immunosuppressive drugs used to treat the malignancies. Unless treated, toxoplasmosis in cancer patients may be lethal. The combination of pyrimethamine, sulfadiazine and folic acid is the treatment of choice (87).

## **TOXOPLASMOSIS AND TRANSPLANTATION**

Transplantation is the transfer of living cells, tissues, or organs from one individual to another. Except in the case of identical twins, the donor's tissue antigens will be seen as foreign by the recipient's humoral and cellular immune system, leading to eventual rejection of the transplant. In order to have a successful transplant of an organ or tissue from one individual to another, it is necessary that the donor and recipient share as many tissue characteristics as possible. These include blood type antigens, major histocompatibility complex (MHC) class I and class II antigens, minor histocompatibility antigens, and non-MHC antigens. The better the match between donor and recipient antigens, the longer the transplanted tissue or organ will survive (1, 10). A second factor necessary for successful transplantation and survival of the grafted tissue is the use of an immunosuppressive drug such as cyclosporin A, which aids in the prevention of graft rejection by inhibiting cell-mediated immunity (1, 10). Clinicians must be aware that toxoplasmosis may be seen in organ transplant recipients either due to reactivation of latent disease or to acquisition of a new disease transmitted by the organ from a *T. gondii*-seropositive individual. In both cases, the use of immunosuppressive therapy triggers the disease (88). Encephalitis is the most common manifestation of toxoplasmosis in immunosuppressed patients (87). It is probably wise to avoid transplantation of *T. gondii* positive organs into seronegative recipients. However, treatment of the recipients with pyrimethamine-sulfadiazine-folic acid will generally control the infection (87).

The most common immunosuppressive drug used in transplant surgery and in treatment of certain autoimmune diseases is cyclosporin A. Cyclosporin A is a nonpolar cyclic oligopeptide fungal metabolite with selective effects on CD4<sup>+</sup> T cells (133, 137). T-cell activation is prevented by cyclosporin A blocking a step in a Ca<sup>2+</sup>-dependent signal transduction pathway necessary for the transcription of cytokine genes (137, 180). Thus, there is inhibition of cytokine secretion with resultant general suppression of the activities of both T-cell and non-T-cell components of the immune system.

In addition to its ability to immunosuppress the mammalian host, cyclosporin A also can inhibit the growth of certain parasites. In vitro, *T. gondii* replication in mouse macrophages is inhibited by cyclosporin A (112, 121). However, the effect of cyclosporin A in vivo is less clear. In some experiments, administration of cyclosporin A protected mice infected by *T. gondii*, and in other experiments, protection was not seen (121). Nonetheless, McCabe et al. (121) recommended the use of cyclosporin A in transplant patients to prevent the reactivation of the parasite.

There are two cyclosporin A-binding proteins in *T. gondii*. These proteins are termed cyclophilins and catalyze the isomerization of peptidylproline bonds (78,

137). Enzyme activity is inhibited by nanomolar levels of cyclosporin A. The role of cyclophilins in *T. gondii* is unknown but cyclophilins may be necessary for virulence.

#### Renal transplants

Derouin et al. (39) studying 73 kidney transplant recipients and Renoult et al. (156) studying 69 recipients concluded that the risk of developing toxoplasmosis from kidney transplantation was quite low. Clinical toxoplasmosis was not seen in pretransplant *T. gondii*-seropositive patients nor in pretransplant seronegative patients who had received an organ from a seropositive donor. Even though an increase in *T. gondii* antibody was noted in a few patients, there was no evidence of clinical symptoms. Nonetheless, a few cases of clinical disease have been seen in kidney seronegative transplant recipients receiving kidneys from positive donors or in recipients whose inactive toxoplasmosis had reactivated (9, 88, 114, 157, 158, 167).

#### Liver transplants

Clinical toxoplasmosis in liver transplantation appears to be a rare event (117). However, a few cases of reactivated toxoplasmosis have occurred in *T. gondii*-seropositive liver transplant recipients (98, 117).

#### Bone marrow transplants

In three studies devoted to the incidence of toxoplasmosis after allogenic bone marrow transplantation, toxoplasmosis was demonstrated in 12 of 3,803 patients (183), in 1 of 482 patients (172) and in 7 of 296 patients (40). The frequency of toxoplasmosis in these reports of 4,581 bone marrow transplant patients ranged from 0.21 to 2.4% (mean = 0.44%); thus, toxoplasmosis appears to be a rare event as an aftermath of bone marrow transplantation. Reactivation of *T. gondii* in seropositive recipients accounted for most of the toxoplasmosis seen during bone marrow transplantation (124, 143, 183). Reactivation was probably due to immunosuppressive drug use. Still, the parasite can be introduced into a seronegative recipient by the bone marrow of a seropositive donor (91).

#### Heart transplants

There appears to be little risk of toxoplasmosis for the seropositive cardiac transplant recipient regardless of donor status. When toxoplasmosis does occur, it is generally acquired by a seronegative recipient from a seropositive donor heart (41, 183). In a study involving 40 heart transplant patients, Luft et al. (105) found that none of 27 *T. gondii*-seronegative recipients who received hearts from seronegative donors developed infection. Toxoplasmosis was not seen in 19 seropositive recipients regardless of donor status; however, 10 of the 19 did show elevation of *Toxoplasma* antibodies. Three of four seronegative recipients who received hearts from seropositive donors developed clinical toxoplasmosis (105). Hakim et al. (69) studied toxoplasmosis in 119 heart transplant patients. There was no disease in 66 seronegative recipients who received hearts from seronegative donors whereas 4 of 7 seronegative recipients of *T. gondii*-seropositive hearts developed toxoplas-

mosis. An additional 7 seronegative recipients receiving seropositive hearts were immediately placed on therapy and did not develop toxoplasmosis (69). Only one of 39 seropositive recipients showed an increase in anti-*Toxoplasma* antibody; he was treated and did not develop toxoplasmosis.

In 21 *T. gondii*-negative heart or heart-lung transplant recipients who received organs from *T. gondii*-positive donors, seven patients did not receive anti-*T. gondii* therapy. Four of these patients developed clinical toxoplasmosis (213). However, in 14 patients who received therapy, only two developed toxoplasmosis. Out of 75 *T. gondii*-seropositive heart or heart-lung recipients, four showed an increase in anti-*T. gondii* titer but no disease (213). In 8 of 15 seronegative recipients receiving hearts from seropositive donors, seroconversion to *T. gondii* was seen in 4 of the 8 patients and 3 of those individuals developed clinical toxoplasmosis (184). Of 26 seropositive heart recipients, six showed a rise in anti-*T. gondii* titer but none had clinical toxoplasmosis (184). In a study of 290 heart and heart-lung transplant recipients, Orr et al. (134) found that 13 seronegative recipients received organs from *T. gondii*-positive donors. These 13 patients received anti-*T. gondii* therapy; none developed clinical toxoplasmosis and only one individual seroconverted.

The studies with heart transplants indicated that seronegative recipients receiving hearts from *T. gondii*-seropositive donors are at serious risk for developing toxoplasmosis. However, chemotherapy was effective in preventing toxoplasmosis in seronegative recipients who received organs from positive donors. Seropositive recipients did not develop toxoplasmosis regardless of donor status.

## TOXOPLASMOIS AND AIDS

Toxoplasmosis is a major opportunistic infection in immunocompromised individuals, particularly those with AIDS. Major areas of the body that may be affected by toxoplasmosis in AIDS patients include the CNS (80, 107), lung (101, 146), and eye (26, 76). Other areas of the body also may be affected (5, 151).

#### Toxoplasmic encephalitis in AIDS

Toxoplasmic encephalitis (TE) is the second most common opportunistic infection of the CNS in AIDS patients and is due, in most cases, to reactivation of dormant *Toxoplasma* tissue cysts in the brain (106, 160). TE occurs in 10 to 50% of *Toxoplasma*-seropositive AIDS patients whose CD4<sup>+</sup> T-cell count decreases below 100/mm<sup>3</sup>. It is not clear why only some of the seropositive AIDS patients develop TE.

The incidence of TE in AIDS patients is directly proportional to the prevalence of *Toxoplasma* seropositivity in the general population. In French adults, 73 to 90% are seropositive to *T. gondii*, compared to 53 to 59% of Swiss adults (155); approximately 30% of United States adults are seropositive (43). Thus, in those populations with a higher seropositivity to *T. gondii*, more toxoplasmosis will be seen in AIDS (and other immunocompromised) patients. In a New York City study conducted by Grant et al. (66), 7.5% of 411 AIDS patients developed TE, whereas in Geneva and

Lausanne, 17.1% of 504 AIDS patients developed TE (155). It is probable that TE incidence in French AIDS patients is even higher. Grant et al. (66) found that 31.6% of AIDS patients in New York City were seropositive for *T. gondii* (130 of 411) but only 23.8% of those seropositive patients developed TE (31 of 130).

HIV-infected adults with IgG antibody to *T. gondii* and CD4<sup>+</sup> count of <100/mm<sup>3</sup> who undergo their first episode of TE should be treated with trimethoprim-sulfamethoxazole (23). Those AIDS patients who survive their first attack of TE have a risk of relapse (i.e., recrudescence) of 30 to 50% if therapy is not continued (160). Therefore, AIDS patients who have had TE should receive lifelong *Toxoplasma*-suppressive therapy of pyrimethamine-sulfadiazine-leucovorin to prevent recurrence of TE (23). Treatment of TE in AIDS patients is expensive. It has been estimated that it costs \$10,379 (1992 dollars) to treat each case of TE (163).

*T. gondii*-seronegative AIDS patients are at risk of *Toxoplasma* infection and TE if they come in contact with tissue cysts or oocysts (23). HIV-infected individuals who are seronegative should be counseled about sources of *Toxoplasma* infection. They should be advised to avoid handling or eating raw or undercooked meats, to wash hands after handling raw meats or gardening, and to wash raw fruits or vegetables. Cat litter boxes should be changed daily (not by the HIV-infected person), pet cats should not be allowed to go outdoors to hunt and cats should not be fed raw or undercooked meats (23). If the CD4<sup>+</sup> T cell count drops below 100/mm<sup>3</sup> in the *T. gondii*-negative HIV-infected person, he should be tested for seroconversion. If *Toxoplasma* seroconversion has taken place, treatment for TE should be initiated (23). Luft and Remington (108) suggested that *Toxoplasma*-seronegative HIV-positive and AIDS patients in countries where the rate of acquiring toxoplasmic infection is high (e.g., Germany, France, Central Africa, and Haiti) should be advised how to prevent infection and should undergo repeated testing for *Toxoplasma* antibodies.

#### *Pulmonary and ocular toxoplasmosis in AIDS*

The lung is second only to the CNS as a major site of toxoplasmic infection in AIDS patients (130, 146). Clinically, *Toxoplasma* pneumonia is similar to that of *Pneumocystis* and X-ray findings show a strong resemblance of toxoplasmic pneumonia to tuberculosis, histoplasmosis, or coccidiomycosis (73). Thus, it is necessary to demonstrate the presence of *T. gondii* in bronchoalveolar lavage fluids or in lung biopsies to make the proper diagnosis. The utilization of trimethoprim-sulfamethoxazole as a primary prophylaxis against *Pneumocystis carinii* infection has led to a decrease in *Toxoplasma* pneumonia in AIDS patients since the drug combination is effective against both parasites (101).

In AIDS patients, ocular toxoplasmosis is less common than cerebral toxoplasmosis (26). Toxoplasmic retinitis in immunocompetent individuals is characterized by areas of necrotizing retinitis, vitreitis, and inflammation of the anterior segment. The lesions usually heal spontaneously. However, in AIDS patients with ocular toxoplasmosis, the retinal lesions are more extensive and blindness may result if left untreated. Signs of systemic toxoplasmosis such as encephalitis,

pneumonitis, and myocarditis may be present with retinitis, also (26, 76). Ocular toxoplasmosis must be differentiated from cytomegalovirus retinitis in order to provide proper treatment (26, 76). The combination treatment pyrimethamine-sulfadiazine-leucovorin will prevent retinitis progression (26). Treatment of the HIV-infected individual must be continued for life to prevent reactivation of the parasite (131).

#### *Factors involved in reactivation of T. gondii*

In *T. gondii*-infected mice, reactivation occurs if there is simultaneous depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and reactivation is related to a decrease in the production of IFN- $\gamma$  and TNF- $\alpha$  (56, 57, 62). In mice with progressive toxoplasmic encephalitis, the CNS response is the induction of the T<sub>H</sub>2 subset of T cells. The T<sub>H</sub>2 response fails to control parasite growth and the characteristic pathology of toxoplasmic encephalitis develops (84). This, too, is probably related to a decrease in IFN- $\gamma$ , since IFN- $\gamma$  is not a cytokine produced by T<sub>H</sub>2 cells. Interestingly, the progression of HIV infection to AIDS leads to loss of IL-2 and IFN- $\gamma$  production concomitant with an increase in production of IL-4 and IL-10 (25). Thus, progression to AIDS causes a T<sub>H</sub>1 switch to a T<sub>H</sub>2 response, thereby allowing the development of opportunistic infections. In *T. gondii*-infected individuals, the lack of T<sub>H</sub>1 cytokines results in reactivation of the parasite with resultant encephalitis (55).

While the T-cell and cytokine changes induced by HIV infection can lead to reactivation of *T. gondii*, TE may exacerbate HIV spread. Activation of brain microglia may be involved in the immune response to TE (169). Microglia are the resident macrophages of the brain; they are capable of phagocytosis, antigen presentation, and cytokine production (195). In patients infected with both HIV and *T. gondii*, activation of HIV-infected microglia by *T. gondii* may stimulate viral replication. Thus, TE may potentiate the intracerebral spread of HIV (169). It is interesting that another opportunistic pathogen in AIDS patients, *Cryptococcus neoformans*, has been reported to potentiate HIV infection (125).

#### **TOXOPLASMOSIS IN THE ELDERLY**

Aging of the immune system alters the ability of the body to control infectious diseases. The elderly ( $\geq 65$  years) are particularly susceptible to opportunistic parasitic and microbial infections (30, 31) and as a consequence, they are probably more susceptible to *T. gondii* infections.

The most dramatic change that occurs in the aging immune system is in the cellular immune component (64). In the aged individual, there is a predominance of the T<sub>H</sub>2 subset of T cells with a corresponding decrease in the T<sub>H</sub>1 subset (64). The T<sub>H</sub>1 cells and their cytokines are the T-cell mediators of primary importance in defense against invasion by intracellular pathogens (93). *T. gondii* is an obligate intracellular pathogen (176) and the T<sub>H</sub>1 response aids the host in resisting infection by the parasite (60, 61, 174, 198). The T<sub>H</sub>2 response is not protective (60, 205). Thus, T<sub>H</sub>1 responses are of major importance in preventing infection by

*T. gondii* and since the elderly are deficient in  $T_H1$  responses, they would appear to be at risk for toxoplasmosis.

Questions that can be asked about toxoplasmosis and the elderly include: are elderly humans more susceptible to *T. gondii* infections? Does reactivation of encysted bradyzoites occur in the *T. gondii*-seropositive elderly?

Aged mice are significantly more susceptible to *T. gondii* infections, with higher mortality than young mice (45, 53, 89). In addition, at similar parasite doses, aged mice had a severalfold increase in brain tissue cyst burden compared to young mice (54). Thus, studies with aged mice would suggest that older humans are more susceptible to *T. gondii* infection and toxoplasmosis but there are no supporting data. However, the prevalence of *T. gondii* seropositivity increases with increasing age in humans (43, 206).

There is no evidence of parasite reactivation in healthy *T. gondii*-seropositive elderly individuals. Interestingly, latent infections of the intracellular pathogens varicella zoster virus and *Mycobacterium tuberculosis* do reactivate in the elderly. Herpes zoster (shingles) results from reactivation of varicella-zoster virus infection acquired in childhood. Reactivated varicella-zoster virus is a disease of older individuals and is correlated with a decrease in  $T_H1$  responses (12, 136, 170). In recent years, tuberculosis has increasingly become a disease of the elderly and generally represents reactivation of a latent infection acquired previously (170, 189). Reactivation of tuberculosis in the elderly is also correlated with a  $T_H1$  decrease. Since shingles, tuberculosis, and toxoplasmosis are caused by intracellular pathogens that require a  $T_H1$  response for control, it is not clear why herpes zoster and mycobacterial infections reactivate in the elderly but *T. gondii* infections do not appear to do so.

## TOXOPLASMOSIS IN IMMUNOCOMPETENT INDIVIDUALS

Postnatally acquired toxoplasmosis in immunocompetent people is generally subclinical and can only be detected immunologically. The infection is acquired by ingestion of tissue cysts from undercooked meats or by inhalation or ingestion of oocysts (43).

### *Toxoplasmic lymphadenopathy*

If the postnatally acquired disease is symptomatic, it most often presents as lymphadenopathy (inflammation of the lymph nodes or glands) with low grade fever, generalized malaise, extreme tiredness, and muscle pain (71). Patients have enlarged lymph nodes in the head and neck regions but lymph nodes in other parts of the body also may be affected (120). *T. gondii* infections have been estimated to cause 3 to 7% of clinically significant lymphadenopathy. *Toxoplasma* lymphadenopathy is generally seen in immunocompetent patients 20 to 40 years of age and may appear similar, clinically and/or histologically, to neoplastic diseases such as lymphoma, Hodgkin's disease, or carcinoma (120). It is important to differentiate toxoplasma lymphadenopathy from neoplastic diseases to avoid unnecessary surgery and/or medication.

McCabe et al. (120) reviewed 107 cases of toxoplasmic

lymphadenopathy in immunocompetent patients. They found that most patients had a disease that involved only one lymph node in the head or neck region and the lymphadenopathy had a benign self-limiting clinical course. However, a few patients demonstrated extranodal disease associated with the lymphadenopathy that included myocarditis, pneumonitis, encephalitis, abnormal liver function, and chorioretinitis. Since transmission of the parasite to the fetus is possible, the most serious extranodal presentation occurs in immunocompetent women who were infected during pregnancy (120). Mawhorter et al. (116) discussed cases of postnatally acquired toxoplasmosis in which cutaneous lesions were a prominent feature of the disease. Therapy is generally not given to immunocompetent patients suffering from toxoplasmic lymphadenopathy unless they have extranodal diseases (122).

### *Toxoplasmic chorioretinitis*

Ocular toxoplasmosis is considered to be the most common infectious disease involving the retina in immunocompetent people. *T. gondii* has been estimated to be the cause of 18 to 55% of all cases of chorioretinitis (122). The peak incidence of ocular toxoplasmosis occurs in the 10- to 35-year-old group (28). Most of the cases are due to reactivation of subclinical prenatal infections (120) but postnatal primary infection may be responsible for some cases (4, 63, 75, 115, 165, 181, 191). Since reactivation of congenital toxoplasmosis can occur years after prenatal infection (97, 211), it can be assumed that most of these congenitally infected individuals are immunocompetent at the time chorioretinitis appears. Thus, because of the probable immunocompetency of these individuals, the reasons for reactivation (tissue cyst rupture) are not understood. In many cases of subclinical congenital toxoplasmosis, ocular disease becomes apparent during adolescence. Roberts et al. (162) have suggested that hormones may trigger retinal tissue cyst rupture, since adolescence is a time of major hormonal changes. However, hormonal changes cannot be the explanation for all cases of reactivated toxoplasmic ocular disease.

Yang et al. (216) attributed the pathological effects of toxoplasmic chorioretinitis to  $CD4^+$  cytotoxic T cells which produced  $IFN-\gamma$ . Cytotoxic cells induced by *T. gondii*-infected retinal melanocytes would destroy infected retinal cells with release of retinal and tachyzoite antigens. The release of the antigens may result in hypersensitivity and/or autoimmune reactions culminating in chorioretinitis (2, 132, 214).

In immunocompetent patients, ocular toxoplasmosis is generally self-limiting and is characterized by necrotizing retinitis, vitreitis, inflammation of the anterior segment, and pigmented chorioretinal scars (26, 168). Even without treatment, the inflammation subsides and within 6 to 8 weeks the lesion heals. However, patients with large retinal lesions and marked vitreitis or patients with lesions in the papillomacular area that threaten vision should be treated (168).

### *Toxoplasmosis of the central nervous system (CNS)*

Most cases of CNS toxoplasmosis are seen in patients with impaired immune systems; however, immunocompe-

tent patients may present with encephalopathy, meningoencephalitis or single or multiple mass lesions (120). The disease may be fatal or the patient may recover partially or fully. Normally toxoplasmosis is self-limiting in immunologically intact patients. Therefore, it is mandatory to check for subtle underlying immunological defects in patients with CNS toxoplasmosis when apparently normal patients are involved (29). Luft and Remington (107), reviewing 31 cases of postnatally acquired encephalitis in people with apparently normal immune systems, found that 3 of 31 patients were infected with another organism in addition to *T. gondii*; 3 of 31 had cirrhosis of the liver; 5 of 31 had received immunosuppressive drugs because of an initial incorrect diagnosis; and 2 of 31 had a history of benign tumors. Another factor that might be involved is age: 4 of the 31 individuals were >60 years of age (mean 67) and may have had depressed immune systems. A thorough clinical study of "normal" CNS toxoplasma patients may indicate that a majority of the patients has subtle nonapparent immune defects which lead to reactivation of the parasite.

#### *T. gondii*-induced Guillain-Barré syndrome

Guillain-Barré syndrome (GBS) is an acute inflammatory demyelinating polyneuritis affecting the peripheral nerves and is marked by paralysis, pain, and wasting muscles. The syndrome has replaced poliomyelitis as the most frequent cause of acute neuromuscular paralysis in developed countries (188). A number of antecedent events appear to trigger GBS, including microbial and viral infections, vaccination, or drugs. Bouchez et al. (15) and Sivertsen and Andersen (182) indicate that infection by *T. gondii* may induce GBS; however, the role of toxoplasmosis in inducing GBS is probably infrequent and of minor importance.

#### *Why clinical toxoplasmosis may occur in immunocompetent individuals*

The pathogenesis of *T. gondii* depends on three factors: the immune status of the host, variation in the virulence of the parasite and the parasite dose. Sibley and Boothroyd (177) and Howe and Sibley (82) showed that virulent *T. gondii* strains have less genetic complexity than would be expected, considering the potential of the parasite for sexual reproduction during its life cycle. Using multilocus restriction analysis on 106 independent isolates from human and animal sources, Howe and Sibley (82) found that there are three clonal lineages of *T. gondii* and that recombination between the three lineages was extremely rare in natural populations. Type II lineage was responsible for approximately two-thirds of clinical toxoplasmosis in humans. Type II strains were often associated with reactivation of chronic toxoplasma infections and account for 65% of cases in AIDS patients (82). Type I strains were usually associated with human congenital toxoplasmosis and type III strains were more commonly associated with animal toxoplasmosis. Since most of the cases of human toxoplasmosis appear to be caused by a single clonal line of *T. gondii*, there is probably little variation in virulence in the strains that make up that clonal line. Therefore, variation in virulence of *T. gondii*

probably does not explain why clinical toxoplasmosis may be seen in immunocompetent individuals.

The data presented by Luft and Remington (107) concerning the questionable immune status of certain "immunocompetent" individuals with toxoplasmic encephalitis would indicate that individuals with toxoplasmic encephalitis may have an underlying defect that renders them less capable immunologically to cope with a *T. gondii* infection. However, a reasonable explanation as to why immunocompetent individuals may have clinical toxoplasmosis would be that they had ingested large numbers of the parasite which simply overwhelmed the immune system's ability to contain the infection.

### TOXOPLASMOSIS AND BLOOD TRANSFUSION

Despite the fact that a large number of potential blood donors are seropositive for *T. gondii*, the only reported cases of transfusion-transmitted toxoplasmosis occurred in patients with acute leukemia on chemotherapy who received leukocytes from *T. gondii*-positive donors with chronic myelogenous leukemia (179). Shulman (175) stated that with current blood-banking procedures, there is less than a one-in-a-million chance of being infected with a parasite from a blood transfusion. Therefore, it is probable that healthy immunocompetent blood donors who are seropositive for *T. gondii* do not transmit toxoplasmosis.

### PERSPECTIVES

Current estimates indicate that approximately one-fifth of the population of the United States suffer from some degree of immune incompetence which may render them more susceptible to opportunistic diseases (Table 1). These individuals include pregnant women, people 65 and over, residents in nursing homes and related care facilities, cancer cases under care, organ transplant patients, and individuals infected with HIV. The deficient immune status of these individuals means that they may be at risk for *T. gondii* infection or recrudescence of a latent infection with ensuing toxoplasmosis.

What can be done to decrease the risk of *T. gondii* infections to immunocompromised populations (and even to

TABLE 1. United States populations with probable deficiency in immune competence

Category (year)	Percent of population affected <sup>a</sup>	Reference
Elderly ≥65 years (1992)	12.66	204 (Table 13)
Pregnant women (1988)	2.49	204 (Table 108)
Cancer cases under care (1992)	1.6	6
Residents in nursing and related care facilities (1991)	0.68	204 (Table 192)
Total HIV infections (Jan. 1993)	0.03–0.05	166
Organ transplant patients (1992)	0.16	204 (Table 190)

<sup>a</sup>Total United States population in 1992 was estimated at 255,082,000 individuals (reference (204), Table 13).

immunocompetent populations)? Programs emphasizing education about *T. gondii* parasitism, protection of the unborn from infection, development of anti-bradyzoite drugs, and development of anti-*T. gondii* vaccines will do much to decrease the incidence of toxoplasmosis.

Education of individuals, starting at an early age, emphasizing the life cycle of *T. gondii*, how the organism is transmitted, and how to prevent infection, should be the first step in the fight against toxoplasmosis. The screening of women of child-bearing age for *Toxoplasma* antibodies should be initiated. The seronegative pregnant woman should be screened for seroconversion and if seroconversion takes place, treatment should be initiated to protect the fetus and the baby during the first year of life. Since relapses are common, the child must be checked regularly for toxoplasmic symptoms and treated, if necessary.

Finally, more emphasis must be placed on the development of anti-bradyzoite drugs and development of vaccines. While there are drugs that are active against the tachyzoite stage of *T. gondii*, none are available that will eliminate tissue cysts containing bradyzoites; thus the individual cannot be cleared of the infection by drugs. Vaccines, particularly of the type that lead to the production of mucosal immunity (IgA), would prevent the infection of intestinal cells by *T. gondii*.

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