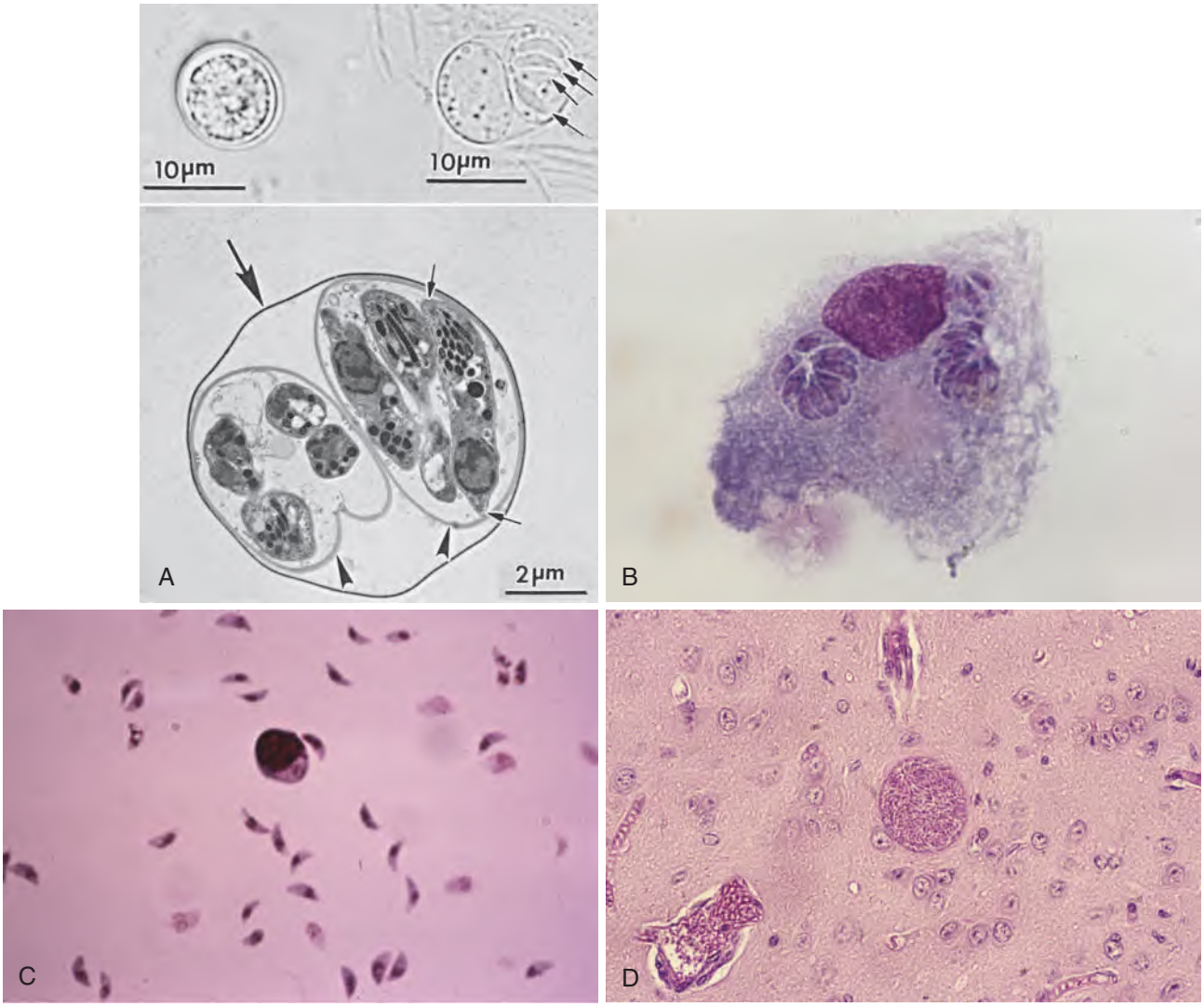

FIG. 278.1 The three forms of *Toxoplasma gondii* observed in nature. (A) Oocysts. Unsporulated oocyst *(top left).* Sporulated oocyst with two sporocysts *(top right).* Four sporozoites *(arrows)* are visible in one of the sporocysts. Transmission electron micrograph of a sporulated oocyst *(bottom).* Note the thin oocyst wall *(large arrow)*, two sporocysts *(arrowheads)*, and sporozoites, one of which is cut longitudinally. (B) Giemsa stain demonstrating two rosettes of intracellular tachyzoites in a mouse bone marrow macrophage. (C), Giemsa-stained smear of mouse peritoneal fluid demonstrating the tachyzoite form. (D) Hematoxylin and eosin stain of the cyst form in brain. *(A courtesy Dr. J.P. Dubey, U.S. Department of Agriculture. Beltsville, MD. B to D courtesy Dr. Jack S. Remington, Stanford University and Palo Alto Medical Foundation.)*

some of the features that make oocysts so robust have come from proteomic and transcriptomic analyses.[30,31] Among other findings, these studies have revealed an abundance of small tyrosine-rich proteins in the oocyst wall. Cross-linking of the tyrosines in these proteins could confer a natural "sun-screen" for the sporozoites within because they are strong absorbers of ultraviolet light.

Tachyzoite

The tachyzoite form (see Fig. 278.1B) is oval to crescentic and measures 2 to 3 μm wide and 5 to 7 μm long; it requires an intracellular habitat to multiply despite having all of the usual eukaryotic machinery necessary for reproduction. Tachyzoites are responsible for triggering immune responses leading to clinical manifestations seen in both primary and reactivated infection; presence of tachyzoites is the hallmark of active infection requiring treatment. They reside and multiply within vacuoles in their host's cells, can infect virtually all phagocytic and nonphagocytic cell types,[16] and multiply approximately every 6 to 8 hours to form rosettes.[32] Continuous multiplication leads to cell disruption and release of organisms that go on to invade nearby cells or are transported to other areas of the body by blood and lymph.[33] Tachyzoites appear to actively and rapidly migrate across epithelial cells and may traffic to distant sites while extracellular.[34] Recent evidence suggests they might also use the infected host cell as a "Trojan horse" to traffic and gain access to tissues that might not otherwise be easily accessed.[35]

At the anterior end of the tachyzoite there is a cone-shaped structure termed the *conoid*. It is protruded during the parasite's entry into host cells. *Rhoptries*, numbering 4 to 12, are club-shaped organelles that terminate within the conoid. The rhoptries, together with surrounding small, rod-shaped organelles (micronemes), have important secretory functions for parasitic invasion. *Dense granules* are organelles distributed throughout the cytoplasm. Their contents are released into a vacuole, termed the *parasitophorous vacuole*, that is formed around the parasite during entry into the cell and also into the external environment as excreted-secreted antigens.[16] Some dense granule proteins transit across the membrane surrounding the parasitophorous vacuole, eventually reaching the host nucleus, as described further.[36]

The rhoptries and micronemes produce a collection of proteins that are crucial for the invasion process.[37] These appear to mediate the attachment to the host cell, including the moving junction, a ringlike point of contact between the parasite and host cell surface that migrates down the length of the parasite during invasion. Like the dense granules, the rhoptries also introduce proteins into the host cell that are critical in manipulating the cell, presumably to the advantage of the parasite.[38] These rhoptry and dense granule proteins can be very different (polymorphic)

between different strains of *T. gondii* and appear responsible for many of the differences in virulence seen for types I, II, and III in mice, as described earlier.

A fourth organelle that is restricted to *Toxoplasma* and its Apicomplexa cousins is the apicoplast.[39] This is akin to chloroplasts of plants, with a similar evolutionary origin involving endosymbiotic algae, but photosynthetic functions have been completely lost. It has its own DNA, RNA, and protein translation, the latter of which is prokaryotic in nature, making for a very attractive target for drug therapies. Indeed, one of the currently used drugs for treatment of human infection, clindamycin, targets the ribosomes of the apicoplast.[40] A major role of this organelle is fatty-acid biosynthesis.[39] Recent work with *Plasmodium,* which also has this organelle, has demonstrated that the apicoplast is crucial to the synthesis of isoprenoids,[41] making further work on attacking this Achilles heel an attractive prospect.

Tachyzoites cannot survive desiccation, freezing and thawing, or extended exposure to gastric digestive juices.[33] They are propagated in the laboratory in the peritoneum of mice and in cultured cells. Tachyzoites can be visualized in sections stained with hematoxylin and eosin (H&E) but are better visualized with Wright-Giemsa and immunoperoxidase stains.[42] They are used as the killing target in the gold standard test for detection of *Toxoplasma* IgG.

Tissue Cyst

Once the tachyzoite has invaded the target cell, it can undergo stage conversion into the bradyzoite form.[16] Tachyzoites and bradyzoites are structurally and phenotypically different. Tachyzoites multiply rapidly and synchronously, forming rosettes and lysing the cell, whereas the more slowly replicating bradyzoites form tissue cysts.[43] Molecules are expressed in a stage-specific manner and are responsible for certain of the phenotypic differences between tachyzoites and bradyzoites. Interferon-γ (IFN-γ), nitric oxide (NO), heat shock proteins, and pH and temperature manipulations can trigger conversion of tachyzoites to bradyzoites in vitro and perhaps in vivo as well.[16]

Tissue cysts grow and remain within the host cell cytoplasm, wherein the intracystic bradyzoites continue to divide. Tissue cysts vary in size from younger ones that contain only a few bradyzoites to older tissue cysts that may contain several thousand bradyzoites and may reach more than 100 μm in size (see Fig. 278.1D). They appear spherical in the brain and conform to the shape of muscle fibers in heart and skeletal muscles. The central nervous system (CNS); eye; and skeletal, smooth, and heart muscles appear to be the most common sites of latent infection.[44] Because of their persistence in tissues in asymptomatic individuals, demonstration of tissue cysts in histologic sections does not necessarily mean that the infection was recently acquired or that it is clinically relevant. Tissue cysts stain well with periodic acid–Schiff, Wright-Giemsa, Gomori methenamine silver, and immunoperoxidase stains. Tissue cysts in meat are rendered nonviable by γ-irradiation (0.4 kGy),[45] heating meat throughout to 67°C, or freezing to –20°C for 24 hours and then thawing,[46,47] but not by gentle heating in a microwave.[48]

Although the tachyzoite form appears to be indiscriminate in the type of host cell parasitized, it has been suggested that, in brain tissue, there is a predilection for tissue cyst formation to occur predominantly within neurons.[49,50] However, it has been shown that tissue cysts can form within astrocytes cultured in vitro.[51] In an electron microscopic study of the pathologic changes in brains of infected mice, tissue cysts were observed to remain intracellular throughout the period of study (22 months).[49] There is compelling evidence to suggest that bradyzoites can exit from intact tissue cysts and invade contiguous cells, where they convert to the tachyzoite form.[52] This is the likely explanation for the appearance of "daughter" cysts or clumps of cysts in the brain. Recent evidence suggests that neurons are capable of destroying the invaded parasite and/or that rhoptry proteins are injected into cells they do not infect.[53] These results have implications for how the parasite commandeers host functions.

TRANSMISSION AND EPIDEMIOLOGY

T. gondii infection is a worldwide zoonosis. The organism infects herbivorous, omnivorous, and carnivorous animals, including birds.[54] Infection in humans most commonly occurs through the ingestion of raw or undercooked meat that contains tissue cysts, through the ingestion of water or food contaminated with oocysts, or congenitally through transplacental transmission from a mother who acquired her infection during gestation (Fig. 278.2). Less common are transmission by transplantation of an infected organ or transfusion of contaminated blood cells. Transmission has also occurred by accidental sticks with contaminated needles[55] or through exposing open lesions or mucosal surfaces to the parasite.[56] Because the sexual cycle of the parasite takes place in the small bowel of members of the cat family, cats play a significant role as powerful amplifiers of the infection in nature (see "Oocyst").[16] Epidemiologic surveys have revealed that in most areas of the world, the presence of cats is of primary importance for the transmission of the parasite. Excretion of oocysts has been reported to occur in approximately 1% of cats in diverse areas of the world.[56] Wild felines, and especially bobcats in the United States, may be among the most important sources of oocysts.[57]

Although ingestion of raw or undercooked meat that contains viable *T. gondii* tissue cysts will result in infection, the relative frequency with which this occurs in relation to the frequency of infection caused by ingestion of oocysts is unclear. For instance, in countries such as France, where eating undercooked meat is common and the prevalence of the infection is high, meat may be an important cause of the infection. (It was in Paris that the meat-to-human hypothesis of spread of *T. gondii* was proved.[58]) In contrast are countries such as those in Central and South America, where the prevalence of the infection in humans is high but the ingestion of undercooked meat is relatively less common. In these areas, oocysts may be the more important source of human infection.[59] Until a newly described method for distinguishing bradyzoite- versus oocyst-initiated infection is validated in these regions,[60] their respective contributions to human infection will largely remain a matter of speculation.

Ingestion of tissue cysts in infected meat (primarily pork and lamb) is a major source of the infection in humans in the United States.[61,62] *T. gondii* infection is common in many animals used for food, especially sheep and pigs, with a lower prevalence in cattle, horses, and water buffaloes. Organisms may survive in tissue cysts in these animals for years and can be found in nearly all edible portions of an animal.[63] A seminal study on the prevalence of *T. gondii* in samples of meat used for human consumption (obtained from grocery stores) was performed in the United States in the 1960s.[64] The parasite was isolated from 32% of pork chops and 4% of lamb chops; there were no isolations from beef, and indeed, cattle appear to be generally not an important intermediate host for this parasite.[65] A recent polymerase chain reaction (PCR)-based study in England found 33% (19/57) of pork and 67% (6/9) of lamb samples positive for *T. gondii* DNA.[66]

Serologic surveys conducted in the past 20 years in the United States indicate that the prevalence of *T. gondii* in pigs is declining, with an overall prevalence of 2.6% in a recent National Animal Health Monitoring Survey,[67] presumably due to changing management practices and consolidation of pig production into large-scale operations. However, there are still many isolated small swine farms, including those that raise organic pigs, and the prevalence of *T. gondii* in these animals can be greater than 90%.[68] Of note, meat for human consumption is not routinely inspected for *T. gondii* infection in the United States or elsewhere in the world.[69] Seroprevalence of *T. gondii* infection in a study of lambs in the mid-Atlantic region was recently reported to be 27%.[70] Although *T. gondii* infection of sheep is widely prevalent, in the United States meat from adult sheep is not usually used for human consumption.[56] Reports of suspect transmission by unpasteurized goat's milk have appeared.[71,72] In addition to differences in how meat is cooked, the tendency for beef, compared with lamb and pork, to harbor few, if any, cysts may partly explain the differences in seroprevalence in the United States versus Europe; beef accounts for a much greater fraction of meat consumed in the United States compared with Europe, where lamb and pork are more popular.

Acute infection appears to exhibit seasonality patterns in studies reported from Europe and the United States.[73] In a study from the Palo Alto Medical Foundation–*Toxoplasma* Serology Laboratory (PAMF-TSL; www.pamf.org/serology/; 650-853-4828) of 112 consecutive cases of

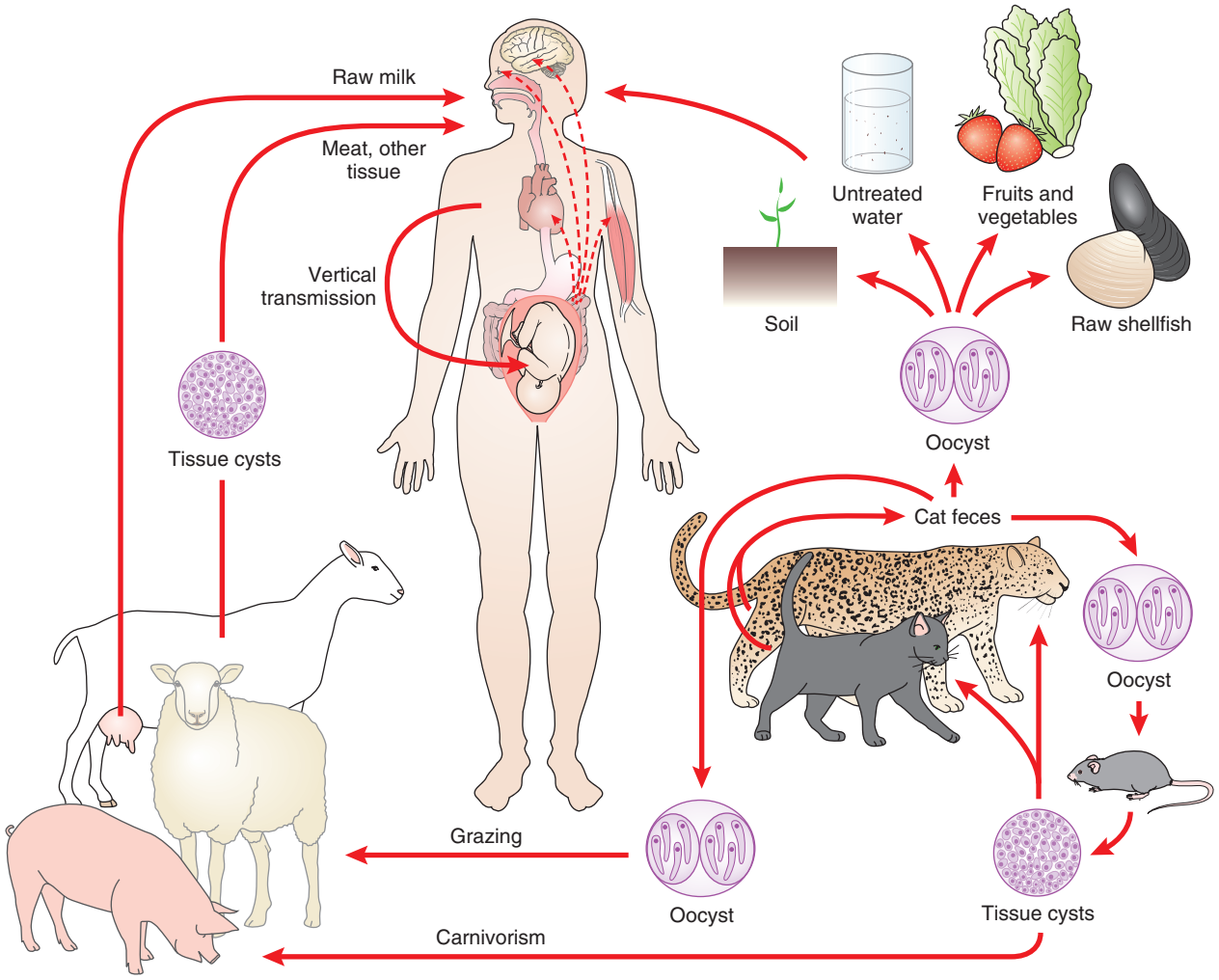

FIG. 278.2 **Transmission and life cycle of *Toxoplasma gondii*.**

acute toxoplasmic lymphadenitis (TL) in the United States, the distribution of cases was not uniform across the 12 calendar months. The highest peak of cases was in December, followed by a peak in September. Similar months were identified in patients with acute toxoplasmosis in rural areas in France.[74]

T. gondii infection is prevalent in game animals, especially black bears (80% infected) and white-tailed deer, as well as in raccoons (60% infected).[63] Thus wild animal meat can serve as a source of the infection for hunters and their families, especially when care is not taken while eviscerating and handling the game or when meat and other organs from these animals is served undercooked or uncooked.[63]

Although *T. gondii* tissue cysts may be found in edible tissues of chickens,[75] poultry products are probably not important in the transmission of *T. gondii* to humans in urban areas because they are usually frozen for storage and thoroughly cooked to avoid diseases that could be caused by contamination by other organisms.[56]

The ingestion of vegetables and other food products contaminated with oocysts probably accounts for infection in seropositive vegetarians. Although isolation of tachyzoites from secretions of people with the acute infection has been claimed, human-to-human transmission of infection by this route has not been established. Outbreaks within families and other groups are common,[76–79] but there is no evidence of natural human-to-human transmission other than from mother to fetus.

Several epidemiologic studies have identified water as a potential source for *T. gondii* infection both in humans and animals.[76,80–83] In vitro studies have demonstrated that oocysts can sporulate in seawater within 1 to 3 days, can survive in seawater for up to 6 months, and can survive in water treated with sodium hypochlorite or ozone, but not ultraviolet radiation.[84–86] Population mapping studies of acutely infected individuals as well as case-control studies linked drinking unfiltered water (presumably contaminated with oocysts) to an outbreak of toxoplasmosis in a municipality in the Western Canadian province of British Columbia[76] and to high endemic rates of toxoplasmosis in Rio de Janeiro State, Brazil.[81] In another Brazilian outbreak, *T. gondii* organisms were detected in water by a variety of methods from an implicated reservoir.[87] Coastal freshwater runoff was observed to be a risk factor for *T. gondii* infection among southern sea otters along the California coast.[88]

In humans the incidence of *T. gondii* antibodies increases with increasing age; the incidence does not vary significantly between sexes. The incidence tends to be less in cold regions, in hot and arid areas, and at high elevations. Slaughterhouse workers may have an increased risk for infection. The prevalence of antibody titers to *T. gondii* varies considerably among different geographic areas and also among individuals within a given population. These differences depend on a variety of factors, including culinary habits and cleanliness of surroundings. A decrease in antibody prevalence over the past few decades has been observed in many countries. In the United States the seroprevalence in US military recruits decreased by one-third between 1965 and 1989; the crude seropositivity rate among recruits from 49 states was 9.5% in 1989 compared with 14.4% in 1965.[89] As another example, in the 1970s, 24% of women in the childbearing age group in Palo Alto, California were seropositive, whereas the rate in 2008 was 10%. Seroprevalence rates in the United States among such women range from 3% to greater than 35%, whereas rates greater than 50% are present in women of childbearing age in much of Western Europe, Africa, and South and

Central America.[90] The recent (2011–14) overall age-adjusted seroprevalence of *T. gondii* infection in persons 6 years or older in the United States was reported to be 10.4%, with a seroprevalence among women age 15 to 44 years at 7.5%. This represented a continuous decline in seroprevalence compared with a similar survey from 15 to 20 years earlier. In multivariable analysis, *Toxoplasma* seroprevalence increased with age and was higher in males; persons living below the poverty level; persons with a high school or less education; and non-Hispanic black, Mexican American, and foreign-born non-Hispanic white persons compared with US-born non-Hispanic white persons.[91]

Although the prevalence of the infection appears to be declining in certain areas of the world, such as Europe and the United States, this has not been the case, or there has been a documented increase, in other geographic locales.[92] A meta-analysis of blood donors worldwide found an overall seroprevalence of 33%, with a wide range among countries.[93]

T. gondii may survive in citrated blood at 4°C for as long as 50 days, and infection has been transmitted through transfusion of whole blood or white blood cells. Leukocyte transfusions may pose a special risk.[94] The transmission of infection by organ transplantation has been documented and may result from the transplantation of an organ (e.g., heart) from a seropositive donor to a seronegative recipient.[95] In bone marrow transplant (BMT) recipients, toxoplasmosis almost always is a result of recrudescence of a latent infection rather than from the transplant.[96,97]

The incidence of TE among HIV-infected individuals directly correlates with the prevalence of *T. gondii* antibodies among the general HIV-infected population, the degree of immunosuppression (best measured by the CD4 cell count),[98] the use of effective prophylactic treatment regimens against development of TE, and the immunologic response to antiretroviral therapy (ART).[99] AIDS-associated TE and toxoplasmosis involving other organs are almost always due to reactivation of a chronic (latent) infection that results from the progressive immune dysfunction that develops in these patients.[100] It is estimated that 20% to 47% of AIDS patients who are infected with *T. gondii* but are not taking anti-*Toxoplasma* prophylaxis or antiretroviral drugs will ultimately develop TE.[98,100] This makes TE a major concern in areas where the use of antiretrovirals is still a treatment relatively few HIV-positive individuals receive.

A substantial decline in the incidence of TE[101] and toxoplasmosis-associated hospitalizations[102] and deaths[103] has been seen in HIV-infected patients who adhere to effective anti-*Toxoplasma* prophylactic regimens and to ART.

In the United States *T. gondii* seropositivity among HIV-infected patients has been reported to range from 10% to 45%[86] and directly correlates with the seropositivity in the general non–HIV-infected population. In contrast, the seroprevalence from a similar period was approximately 50% to 78% in certain areas of Western Europe and Africa.[104,105] In a study in France, 1215 (72.2%) of 1683 HIV-infected patients had serologic evidence of exposure to *T. gondii*.[106] During the study period (1988–95), the overall incidence of toxoplasmosis in this population was estimated to be 1.53 per 100 patient-years, with an increase from 0.68 per 100 patient-years in 1988 to 2.1 per 100 patient-years in 1992, and a subsequent decline to 0.19 per 100 patient-years in 1995 that was likely related to the widespread use of anti-*Toxoplasma* prophylaxis. Toxoplasmosis is rare in the HIV-infected pediatric population: 0.06 cases per 100 patient-years were reported among more than 3000 patients participating in clinical trials in the pre-ART era but during a time when *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis was recommended.[107]

Of interest is the low reported incidence of TE in Africa, despite *T. gondii* seroprevalence rates of 32% to 78%. Lack of autopsy data and a lack of neuroimaging studies likely contribute to the low reported incidence. It has also been suggested that because of poor access to medical care, many HIV-infected patients in Africa succumb to infection with organisms such as *Mycobacterium tuberculosis* before they develop the opportunistic infections associated with the advanced stage of HIV infection, including toxoplasmosis. However, in one autopsy series from the Ivory Coast, of 175 patients with AIDS-defining abnormalities, the prevalence of TE was 21%,[108] and in a recent report of 547 HIV-infected patients from Ghana, TE accounted for 29% of admissions and 28% of deaths.[109]

T. gondii infection may be acquired after the acquisition of HIV infection. Seroconversion rates between 2% and 5.5% have been reported in patients followed for periods up to 28 months.[110]

Even before the emergence of AIDS, TE had been recognized as a cause of incapacitating disease and death among HIV-negative immunosuppressed patients,[6,111] especially in those whose underlying disease or therapy caused a deficiency in cell-mediated immunity. Patients with hematologic malignancies are at a particularly higher risk to develop recrudescence of the infection.[112] Among organ transplantation patients, those with heart, lung, kidney, and BMTs develop toxoplasmosis at a higher rate.

PATHOGENESIS AND IMMUNITY

T. gondii multiplies intracellularly at the site of invasion, with the gastrointestinal [GI] tract as the major route for and the initial site of infection in nature; bradyzoites released from tissue cysts or sporozoites released from oocysts invade, differentiate to tachyzoites, and then rapidly multiply within intestinal epithelial cells. Organisms may spread first to the mesenteric lymph nodes and then to distant organs by invasion of lymphatics and blood. *T. gondii* tachyzoites infect virtually all cell types, and cell invasion occurs as an active process. Survival of tachyzoites is due to the formation of a parasitophorous vacuole that lacks host proteins necessary for fusion with lysosomes,[113] and consequently acidification does not occur. Active invasion of macrophages by tachyzoites does not trigger oxidative killing mechanisms. With the appearance of humoral and cellular immunity, only those parasites protected by an intracellular habitat or within tissue cysts survive. An effective immune response significantly reduces the number of tachyzoites in all tissues, and after the initial acute stages, tachyzoites are rarely demonstrable histologically in tissues of infected immunocompetent humans. Tachyzoites are killed by reactive oxygen intermediates,[114] acidification,[115] osmotic fluctuations, reactive nitrogen intermediates,[116] intracellular tryptophan depletion,[117] and specific antibody combined with complement.[118] In rodents two classes of immune-stimulated guanosine triphosphate (GTP)ases play a crucial role in destruction of tachyzoites within the parasitophorous vacuole: the p47 immunity-related GTPases (IRGs)[119] and the larger GTP-binding proteins (GBPs).[120]

Tissue cyst formation takes place in multiple organs and tissues during the first week of infection. Despite the ability to isolate *T. gondii* from normal brains of chronically infected humans, the tissue cyst form is rarely observed in histologic preparations; it has been isolated from both brain and skeletal muscle in 10% of 52 *T. gondii*–seropositive patients who, at autopsy, had no clinical or pathologic evidence of the infection.[44] The tissue cyst form is responsible for residual (chronic or latent) infection and persists primarily in the brain, skeletal and heart muscle, and the eye.[44,121]

In immunocompetent individuals the initial infection and the resultant seeding of different organs leads to a chronic or latent infection with little, if any, clinical significance. This chronic stage of the infection corresponds to the asymptomatic persistence of the tissue cyst form in multiple tissues. It is believed that periodically bradyzoites are released from tissue cysts or that cysts "rupture"; cyst disruption in this setting appears to be a clinically silent process effectively contained by the immune system and in the CNS likely results in small inflammatory nodules, with a limited degree of neuronal cell death and architectural damage.[52] However, several investigators have suggested that chronic infection may not be completely asymptomatic and may result in important behavioral changes and neuropsychiatric disorders,[122–124] although other studies do not have similar findings, and definitive research to support such associations has not yet been reported.

Toxoplasmosis in severely immunodeficient individuals may be caused by primary infection or be the result of recrudescence of a latent infection. It is widely held that reactivation is the result of disruption of the tissue cyst form, followed by differentiation to and uncontrolled proliferation of tachyzoites and tissue destruction. In individuals with deficient cell-mediated immunity, rapid, uncontrolled proliferation of *T. gondii* results in progressively enlarging necrotic lesions. It has been postulated that damage to any organ in these patients, including the

brain, eye, heart, lung, skeletal muscle, GI tract, and pancreas, can result directly from tissue cyst disruption in the parenchyma of the organ itself or from tissue cyst disruption elsewhere in the body, followed by subsequent spread to that organ.[125] Hematogenous spread is supported by the observation of the development of simultaneous lesions in the brain and the presence of parasitemia in 14% to 38% of AIDS patients with TE.[126,127]

Infection with *T. gondii* induces both humoral and cell-mediated immune responses. A well-orchestrated and effective systemic immune response, combining both innate and adaptive mechanisms, is responsible for the early disappearance of *T. gondii* from peripheral blood during the acute infection and limits the parasite burden in other organs. Immunity in the immunocompetent host is lifelong. Exogenous reinfection, which has been demonstrated in laboratory animals, likely also occurs in humans but does not appear to result in clinically apparent disease, although in one case report a chronically infected pregnant woman was infected with a highly virulent strain that resulted in infection of the fetus.[128]

Because *T. gondii* is a natural parasite of rodents, inbred mice have been used extensively as an animal model for studies of both immunity and immunopathology in this protozoan infection and have yielded a remarkably detailed picture of its host interaction.[129–131] When tachyzoites invade, they inject the contents of their rhoptries into the host cell cytosol. This delivers not only some of the machinery needed for invasion (contained within the rhoptry necks and known as RON proteins) but also a collection of "effectors" that intercept or co-opt host immune pathways, presumably to the parasite's advantage. These effectors include the rhoptry protein ROP16, which functions as a mimic of host Janus kinases (JAKs), phosphorylating critical tyrosines on host STATs (signal transducers and activators of transcription). Depending on the particular flavor of ROP16 that a given strain carries, the host immune response can be driven in differing directions, either more or less inflammatory, and this can have a profound effect on the host's response and ultimate outcome of the infection. In the case of another set of injected ROPs—ROP5, ROP17, and ROP18—the target is murine IRGs, a key part of a mouse cell's defense machinery.[132] Normally, IRGs attack the membrane of the vacuole in which a pathogen resides, disrupting it and leading to death of the organisms within. ROP5, ROP17, and ROP18, however, collaborate to phosphorylate and thereby inactivate IRGs, although again, the effectiveness of this depends on the specific alleles of ROP5 and ROP18 that a given strain of *Toxoplasma* carries. Last, dense granules can also introduce polymorphic effectors into the host cell; one such effector, granule 15 (GRA15), has been shown to be crucial to the activation of one of the most central transcription factors in mammalian immune response, nuclear factor kappa B.[133]

Another, GRA6, activates nuclear factor of activated T cells (NFAT) activity.[134] Some GRA proteins transit across the parasitophorous vacuole membrane (PVM) that separates the growing parasites from the host cytosol, eventually reaching the host nucleus. These include GRA16,[135] GRA24[136] and TgIST (*T. gondii* inhibitor of STAT1 transcriptional activity),[137,138] all of which have profound effects on how the host cell responds to infection. In the case of TgIST, for example, the secreted protein suppresses the immune response by blocking the activity of a key host factor STAT1. These proteins transit across the PVM by an as yet uncharacterized machinery, although one component was recently described (myc regulation 1 [MYR1]).[139] It is important to recognize that these findings do not necessarily represent the mechanisms underlying the immune response to *T. gondii* in humans. For example, although the effect of ROP16 on STATs may well have a parallel in human cells, IRGs are not part of the human immune response, and so ROP5, ROP17, and ROP18, if they are impacting the outcome of human infection, must be doing so by acting on other targets, such as activating transcription factor-6β.[140] Similarly, Toll-like receptors TLR11 and TLR12 play a major role in the induction of interleukin-12 (IL-12) and host resistance in the mouse, but neither receptor exists in humans, indicating that innate recognition of the parasite in humans must involve distinct mechanisms.[141] This might involve another branch of the innate immune system, NLRs (nucleotide oligomerization domain [NOD]-like receptors), that detect molecular signatures or patterns specific to various pathogens. Recent work has suggested that susceptibility to *Toxoplasma* infection in humans is associated with a polymorphism in a human NLR known as NALP1 (NACHT-LRR-PYD domains–containing protein 1).[142] The overall question of how *Toxoplasma* is sensed by the innate arm of the human immune system and how different strains of the parasite do this with differing degrees of success is just beginning to be explored. Of interest, in direct contrast to murine innate cells, human monocytes and dendritic cells do not produce cytokines in response to soluble tachyzoite antigens due to the absence of TLR11 and TLR12. Instead, in human cells phagocytosis of live parasites appears to be a major stimulus for proinflammatory cytokine production.[143]

In murine models T cells, macrophages, and type 1 cytokines (IFN-γ, IL-12) are crucial for control of *T. gondii* infection. Adoptive transfer and depletion experiments not only confirmed that T cells are essential for control of *T. gondii* infection but also demonstrated an interplay between $CD4^+$ and $CD8^+$ T cells in both the induction of resistance and the maintenance of latency. Expansion of both natural killer (NK) and γδ T cells early in infection provides innate resistance while the adaptive response mediated through αβ CD4 and CD8 T cells develops. These different subsets of T cells and NK cells are likely to protect the host by secreting cytokines, such as IFN-γ, IL-2, and TNF-α, and apparently not by lysing *T. gondii*–infected cells.[144–147] Dendritic cells and inflammatory monocytes also play an important role in control of acute infection and the early production of IL-12, with dendritic cells critically dependent on the Fms-like tyrosine kinase 3 (Flt3) ligand for this response.[147–149] NK-cell–derived IFN-γ appears critical to the differentiation of IL-12–producing dendritic cells.[147] Early studies suggested that neutrophils were an important component of the early response, but more recent studies suggest that they are not and may, in fact, contribute to the pathology.[148]

The costimulatory molecules CD28 and CD40 ligand are pivotal for the regulation of IL-12 and IFN-γ production in response to the parasite.[150] *T. gondii* infection of antigen-presenting cells, such as dendritic cells and macrophages, causes upregulation of the counterreceptors for CD28 and CD40L, CD80/CD86, and CD40.[150] Binding of CD80/CD86 to CD28 enhances production of IFN-γ by $CD4^+$ T cells. In addition, binding of CD40L to CD40 triggers IL-12 secretion, which in turn enhances production of IFN-γ. The relevance of CD40L in the immune response to *T. gondii* is supported by reports of TE and disseminated toxoplasmosis in children with congenital defects in CD40L signaling (hyper-IgM syndrome).[151] Moreover, recent studies have demonstrated that expression of CD40L is defective on $CD4^+$ T cells from HIV-infected patients.[152] This deficiency may play a role in defective IL-12/IFN-γ production associated with HIV infection.

Cytokines play a critical role in defense against the infection and are important in the pathogenesis of toxoplasmosis and TE.[153] IL-12 enhances survival of T-cell–deficient mice during *T. gondii* infection, by stimulating the production of IFN-γ by NK cells,[154] and is thought to also regulate the expression of the latter cytokine by T cells in immunocompetent mice.[155] IFN-γ has been shown to play a significant role in the prevention or development of TE in mice.[156] The administration to chronically infected mice of a monoclonal antibody against IFN-γ resulted in a dramatic worsening in the degree of encephalitis.[157] In mice with active TE, treatment with IFN-γ significantly reduced the inflammatory response and numbers of tachyzoites.[158]

Differences in IL-12 levels elicited during infection by different strains of the parasite may be responsible for some of the strain-specific differences in virulence in mice.[159] These differences appear to be related to the activation (phosphorylation) of the transcription factor STAT3, which in turn is dependent on the particular allele of ROP16 injected by a given strain, as detailed earlier.[160]

TNF-α is another cytokine pivotal for control of *T. gondii* infection. TNF-α is required for triggering of IFN-γ–mediated activation of macrophages for *T. gondii* killing activity[161] and for nitric oxide (NO, an inhibitor of *T. gondii* replication) production by macrophages.[162] The administration of TNF-α neutralizing antibody to infected mice caused the death of the mice and an increase in the number of *T. gondii* tissue cysts in the brains of survivors.[163]

IL-10 has been shown to deactivate macrophages and result in reduced in vitro killing of *T. gondii.* Nevertheless, infected IL-10–deficient mice rapidly succumb to proinflammatory tissue damage, indicating an

important protective role for this cytokine.[164] Similarly, IL-4 and IL-6, which are usually considered downregulatory cytokines, have been shown to be important in resistance against TE in the murine model.[165,166] IL-7 has also been shown to have a protective role against *T. gondii* in mice.[167] During the early stages of the infection, IL-12, IL-1, and TNF act in concert with IL-15 to stimulate NK cells to produce IFN-γ.[168]

IL-17[169] and IL-23[170,171] have also been implicated in the generation of a potent immune response but are not thought be essential for host resistance.

Several hypotheses have been proposed to explain the role of IFN-γ in host resistance to *T. gondii*. Involvement of reactive nitrogen intermediates (including NO) is suggested by the observation that L-NG-monomethyl-L-arginine acetate (L-NMMA), a competitive analogue of L-arginine, simultaneously inhibits NO synthesis and intracellular tachyzoite killing by cytokine-activated peritoneal macrophages and microglial cells.[116,172,173] In addition, mice in which NO synthesis is impaired as a result of genetic disruptions of the *IFN-γ* or *IFN-1* genes succumb to the acute infection.[174,175] Similar enhanced susceptibility was observed in mice treated with the reactive nitrogen intermediate inhibitor aminoguanidine[176] and in NO synthase–deficient mice.[177] The protective role of NO appears to be tissue specific rather than systemic.[177] Because control of the acute infection in vivo was unaffected by NO synthase deficiency, the major role of reactive nitrogen intermediates appears to be to maintain control of established infections in this mouse model.[177]

The IFN-γ–inducible p47 GTPases IRGM3 (IGTP) and IRGM1 (LRG47) have been shown to be required for host control of *T. gondii* infection in the mouse,[178] and recent studies have linked IRGM3 with the autophagic destruction of *Toxoplasma*-containing vacuoles in IFN-γ–activated macrophages.[179,180]

In a recent cytokine study using a high-throughput and multiplex assay, C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10 levels were higher ($P < .05$) and resistin levels were lower ($P < .05$) in pregnant women with TL compared with those without TL. Among patients with TL, levels of vascular cell adhesion molecule 1 (VCAM-1) and C-C motif chemokine ligand 2 (CCL2) were lower ($P < .05$) in pregnant women than in nonpregnant women.[181] These findings likely illustrate human immune responses and parasite subversion efforts to strike a balance among immune protection, pathology, and evasion.

Immunoglobulin G (IgG), IgM, IgA, and IgE antibodies are produced in response to the infection. Extracellular tachyzoites are lysed by specific antibody when combined with complement; this is the basis for the detection of *Toxoplasma* IgG in what is considered to be the gold standard test for this Ig. In mice, humoral immunity results in limited protection against less virulent strains of *T. gondii* but not against virulent strains.[182]

Both astrocytes and microglia likely play important roles in the immune response against *T. gondii* within the CNS. In the early stages of TE in both humans and mice there is a remarkable and widespread astrocytosis restricted to areas in which the parasite is detected.[153] Whereas *T. gondii* can invade, survive, and multiply within astrocytes, they are killed by activated microglia.[183]

GENETIC SUSCEPTIBILITY

The observations in mice that genetic factors in the host contribute to the development and severity of TE[184–186] and the fact that not all HIV-infected patients with positive *T. gondii* serologic findings develop TE suggested the possibility that genetic factors may also play a role in the predisposition of AIDS patients for this disease.[187] The major histocompatibility complex (MHC) class II gene *DQ3 (HLA-DQ3)* has been significantly associated with the development of TE in North American white AIDS patients, whereas the *HLA-DQ1* genotype was marginally protective.[187] The *HLA-DQ3* genotype was also significantly associated with the development of hydrocephalus in children with congenital toxoplasmosis.[188] In the latter study a mouse model transgenic for human class II MHC found higher organism burden with the *HLA-DQ3* than *HLA-DQ1* genotype. In a South American white population a study using a higher-resolution typing method identified *HLA-DQB*0402* and *HLA-DRB1*08* genes, which were in linkage disequilibrium, as risk factors for TE, whereas alleles of *HLA-DQB3* were not.[189] Certain *HLA-DQA1* and *HLA-DQB1* alleles were associated with congenital infection in one recent Brazilian study.[190] Another Brazilian study identified an association between the presence of certain KIR receptors and their class I HLA ligands with the development of ocular lesions in *Toxoplasma*-seropositive individuals.[191] Thus further studies are needed to better define the contribution of various HLA alleles to susceptibility to TE. In single studies polymorphisms in other genes, including those for IL-6, IL-10, TLR9, NLR family, pyrin domain–containing protein 1 (NALP1, also known as NLRP1), and purinergic receptor P2X(7) (P2RX7) have been associated with congenital toxoplasmosis or retinochoroiditis.[142,192–195]

Of interest, although humans with genetic defects in IFN-γ and IL-12 signaling are highly susceptible to mycobacterial and other bacterial infections, there are no reports of toxoplasmosis in these patients, again suggesting important differences in how *T. gondii* is controlled in humans versus mice.[196]

PATHOLOGY

Our knowledge of the pathology of infection in humans has come largely from autopsy studies in severely infected infants and immunodeficient patients. Data from immunocompetent adults are limited almost entirely to results obtained from lymph node biopsy specimens[197] and occasionally from myocardial or skeletal muscle tissue specimens.[198] In addition to the direct demonstration of parasites in tissue and associated pathology, recent studies have revealed that the impact of the parasite may extend to many more cells than are actively infected; that is, mouse studies have shown evidence of injected rhoptry proteins in upward of 50-fold more neurons in a chronically infected brain than actually harbor parasites at that time.[53] The impact of these "injected-uninfected" cells on pathogenesis and other interactions with the host has yet to be determined.

Lymph Node

The histopathologic changes in TL in immunocompetent individuals are frequently distinctive and often diagnostic (Fig. 278.3A).[197] There is a typical triad of findings: a reactive follicular hyperplasia, irregular clusters of epithelioid histiocytes encroaching on and blurring the margins of the germinal centers, and focal distention of sinuses with monocytoid cells (see Fig. 278.3A).[199] Langerhans giant cells, granulomas, microabscesses, and foci of necrosis are not typically seen. Rarely, tachyzoites or tissue cysts are demonstrable. *T. gondii* DNA has infrequently been amplified from lymph node tissue.[200]

Central Nervous System

Damage to the CNS by *T. gondii* is characterized by multiple foci of enlarging necrosis and microglial nodules.[201] Necrosis is the most prominent feature of the disease because of vascular involvement by the lesions. In cases of congenital toxoplasmosis, necrosis of the brain is most intense in the cortex and basal ganglia and at times in the periventricular areas.[9,202] The necrotic areas may calcify and lead to striking radiographic findings suggestive but not pathognomonic of toxoplasmosis. Hydrocephalus may result from obstruction of the aqueduct of Sylvius or foramen of Monro. Tachyzoites and tissue cysts may be seen in and adjacent to necrotic foci, near or in glial nodules, in perivascular regions, and in cerebral tissue uninvolved by inflammatory change.[203] The necrotic brain tissue autolyzes and is gradually shed into the ventricles. The protein content of such ventricular fluid may be in the range of grams per deciliter and has been shown to contain significant amounts of *T. gondii* antigens.

The presence of multiple brain abscesses is the most characteristic feature of TE in severely immunodeficient patients and is particularly characteristic in patients with AIDS.[6,204] Brain abscesses in AIDS patients are characterized by three histologic zones. The central area is avascular. Surrounding this is an intermediate hyperemic area with a prominent inflammatory infiltrate and perivascular cuffing by lymphocytes, plasma cells, and macrophages. Many tachyzoites and, at times, tissue cysts as well, appear at the margins of necrotic areas. An outer peripheral zone contains *T. gondii* tissue cysts.[205] In the areas around the abscesses, edema, vasculitis, hemorrhage, and cerebral infarction secondary to vascular involvement may also be present.[206] Important associated features in TE are the presence of arteritis, perivascular cuffing, and astrocytosis.

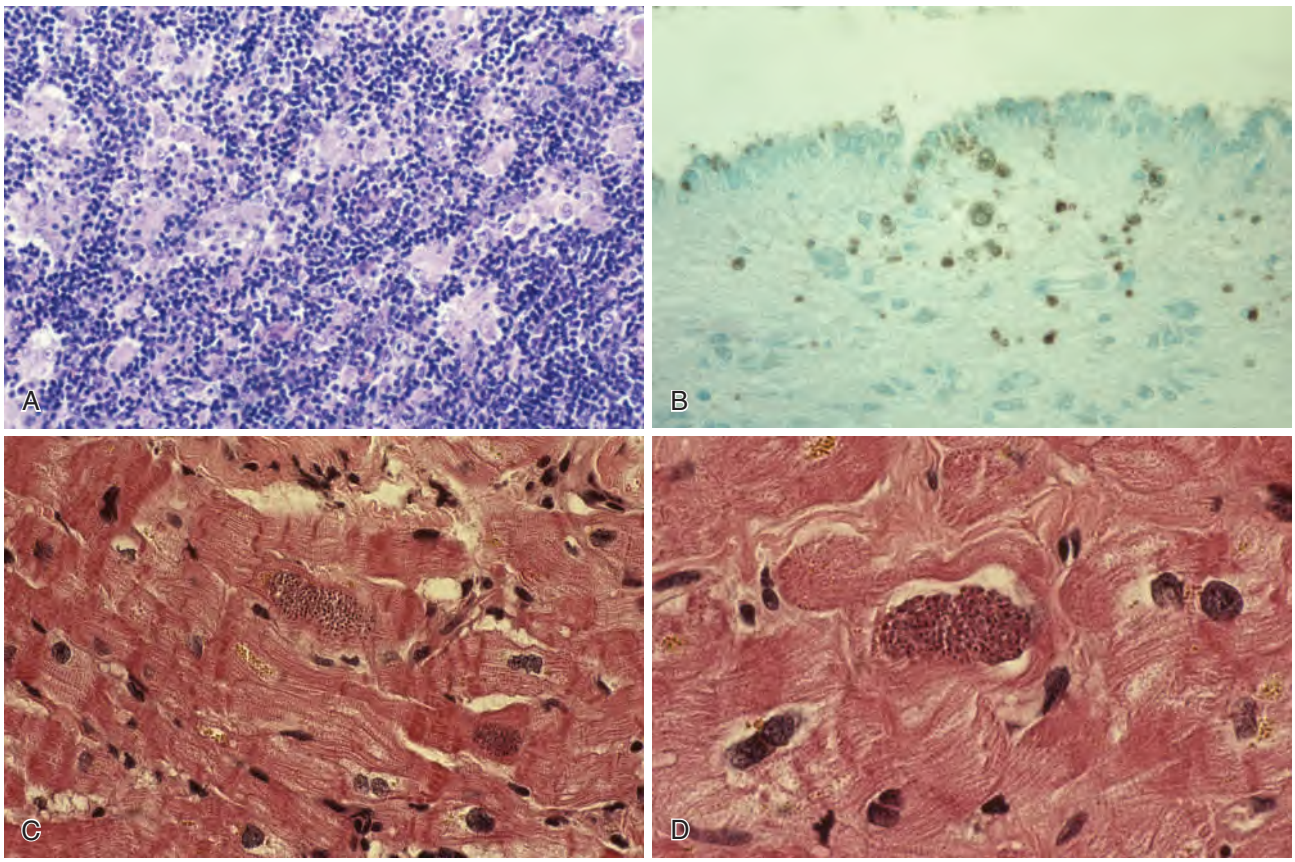

FIG. 278.3 Histologic features of *Toxoplasma gondii* in humans. (A) Hematoxylin and eosin (H&E) stain of a lymph node biopsy specimen from an immunocompetent patient with toxoplasmic lymphadenitis. (B) Positive immunoperoxidase stain of a brain biopsy specimen in a patient with acquired immunodeficiency syndrome and toxoplasmic encephalitis. (C) H&E stain of a right ventricle endomyocardial biopsy specimen from a patient with toxoplasmic myocarditis. Organisms are seen within myocytes (see text under "Clinical Manifestations"). (D) H&E stain of a right quadriceps muscle biopsy specimen depicting tissue cyst from the same patient as shown in C. She also developed toxoplasmic polymyositis. *(A courtesy Dr. Henry Masur, Critical Care Medicine Department, National Institutes of Health, Bethesda, MD. B to D courtesy Dr. Jack S. Remington, Stanford University and Palo Alto Medical Foundation.)*

Because these findings may also be present in patients with viral encephalitis, immunoperoxidase staining is important for differentiating these pathologic processes. Widespread, poorly demarcated, and confluent areas of necrosis with minimal inflammatory response are seen in some patients.[206] Identification of tachyzoites is pathognomonic of active infection, but their visualization may be difficult in H&E-stained sections. The use of immunoperoxidase staining markedly improves the identification of both tissue cyst and tachyzoite forms and highlights the presence of *T. gondii* antigens (see Fig. 278.3B).[42] *T. gondii* DNA can be amplified from cerebrospinal fluid (CSF) or brain biopsy specimens of patients with TE.[207] Of note, PCR-positive results in brain biopsy specimens need to be interpreted with caution. These results may be positive in patients chronically infected with the parasite whose CNS pathology can be explained by a diagnosis other than TE.

At autopsy in AIDS patients with TE, there is almost universal involvement of the cerebral hemispheres and a remarkable predilection for the basal ganglia.[100] In a consecutive autopsy study of 204 patients who died of AIDS, 46 (23%) had morphologic evidence of cerebral toxoplasmosis.[206] In 38 (83%) of the 46 cases, histologic evidence of toxoplasmosis was restricted to the CNS. The cerebral hemispheres were affected in 91% of cases and the rostral basal ganglia in 78%.

A "diffuse form" of TE has been described with histopathologic findings of widespread microglial nodules without abscess formation in the gray matter of the cerebrum, cerebellum, and brainstem.[208] In these patients involvement by *T. gondii* was confirmed by immunoperoxidase stains that demonstrated tissue cysts and tachyzoites. In diffuse TE the clinical course progresses rapidly to death. It has been postulated that in such cases the lack of characteristic findings on computed tomography (CT) or magnetic resonance imaging (MRI) studies is due to insufficient time for abscesses to form before death occurs.

Leptomeningitis is infrequent and, when present, occurs over adjacent areas of encephalitis. Spinal cord necrotizing lesions are seen at autopsy in approximately 6% of patients with TE. The differential diagnosis of TE lesions includes CNS lymphoma, progressive multifocal leukoencephalopathy, pyogenic brain abscesses, and infection caused by organisms such as *Nocardia* spp., *Aspergillus* spp. and other molds, *Trypanosoma cruzi*, *Cryptococcus neoformans, M. tuberculosis, Balamuthia* spp., and cytomegalovirus (CMV). More than one agent may be present.

Lung

Pulmonary toxoplasmosis in the immunodeficient patient may appear in the form of interstitial pneumonitis, necrotizing pneumonitis, consolidation, pleural effusion, empyema, or all of these.[209] The pneumonitis is associated with the development of fibrinous or fibrinopurulent exudate. Tachyzoites may be found in alveolocytes, alveolar macrophages, or pleural fluid, or extracellularly within alveolar exudate. *T. gondii* DNA may be demonstrated in bronchoalveolar lavage (BAL) fluid by the PCR.[210]

Eye

Chorioretinitis in AIDS patients is characterized by segmental panophthalmitis and areas of coagulative necrosis associated with tissue cysts and tachyzoites.[211] Numerous organisms in the absence of remarkable inflammation may be seen around thrombosed retinal vessels adjacent to necrotic areas. Multiple and bilateral lesions may occur.[211] Amplification of parasite DNA in both aqueous humor and vitreous fluid has confirmed or supported the diagnosis of toxoplasmic chorioretinitis in patients

with atypical retinal findings for ocular toxoplasmosis or who are immunocompromised.[212,213]

Eye infection in immunocompetent patients produces acute chorioretinitis characterized by severe inflammation and necrosis.[211] Granulomatous inflammation of the choroid is secondary to the necrotizing retinitis. There may be exudation into the vitreous or invasion of the vitreous by a budding mass of capillaries. Although rare, tachyzoites and tissue cysts may be demonstrated in the retina. The pathogenesis of recurrent chorioretinitis is controversial. One school proposes that rupture of tissue cysts releases viable organisms that induce necrosis and inflammation, whereas another school contends that chorioretinitis results from a hypersensitivity reaction triggered by unknown causes.[211] A study demonstrating efficacy of trimethoprim-sulfamethoxazole (TMP-SMX) in preventing recurrences of chorioretinitis is consistent with the hypothesis that active organism replication is necessary for recurrence.[214]

Recent studies have revealed a much higher incidence of ocular disease, which is often severe, among infected immunocompetent persons in South America than in North America or Europe.[215–218] This difference seems most likely to be due to differences in the strains of *Toxoplasma* that predominate in these different regions.[25] This is consistent with observations within the United States, where specific strains appeared to be associated with severe disease, although these studies involved relatively few patients and cannot be considered definitive.[219]

Skeletal and Heart Muscle

Myositis caused by *T. gondii* has been reported in as high as 4% of HIV-infected patients who present with neuromuscular symptoms, and the same percentage has been observed in autopsy series of AIDS patients in whom a systematic histologic evaluation of the skeletal muscle was performed.[220] Successful isolation from skeletal muscle biopsies has been reported.[221] Microscopy has revealed necrotic muscle fibers with a variable inflammatory reaction. Skeletal muscle involvement has also been reported in the non-AIDS immunodeficient patient.[6,198]

Toxoplasmic myocarditis is frequently noted at autopsy in AIDS patients but is usually clinically inapparent,[222] with CNS manifestations predominating.[223] Focal necrosis with edema and an inflammatory infiltrate is typical,[222] although abscesses may also be noted.[222,223] Similar histologic findings are seen in the non-AIDS immunodeficient population,[6] and in both groups cardiac myocytes may be packed with tachyzoites (to produce pseudocysts) in the absence of an inflammatory response.

Biopsy-proven toxoplasmic myocarditis and polymyositis in the setting of acute toxoplasmosis have been reported in otherwise immunocompetent individuals and in patients on corticosteroids (see Fig. 278.3C and D).[198]

Other Organ Systems

Extensive involvement of the GI tract in AIDS patients may occur with tremendous variation in the inflammatory response.[224,225] Hemorrhagic gastritis and colitis have been described.[226] Other organs reported to be involved during toxoplasmosis include the liver,[227] pancreas,[228] seminiferous tubules,[229] prostate,[229] adrenal glands,[230] kidneys,[231] and bone marrow.[232]

CLINICAL MANIFESTATIONS

Toxoplasmosis describes the clinical or pathologic disease caused by *T. gondii* and is distinct from *T. gondii* infection, which is asymptomatic in the vast majority of immunocompetent patients.

Toxoplasmosis is conveniently classified into five clinical categories: (1) acquired in the immunocompetent patient, (2) acquired or reactivated in the immunodeficient patient, (3) ocular, (4) in pregnancy, and (5) congenital. In any category the clinical presentations are not specific for toxoplasmosis, and a wide differential diagnosis must be considered for each clinical syndrome. Furthermore, methods of diagnosis and interpretation of test results may differ according to the patient's specific clinical category. For instance, whereas serologic test results consistent with an infection acquired in the distant past for a nonimmunocompromised pregnant woman in her first half of pregnancy are interpreted as no risk for congenital toxoplasmosis, the same results for a patient about to undergo an allogeneic hematopoietic stem cell transplantation (HSCT) are interpreted as high risk for life-threatening toxoplasmosis in the posttransplantation period.

Toxoplasmosis in the Immunocompetent Patient

In the United States and Europe only 10% to 20% of cases of *T. gondii* infection in immunocompetent adults and children are symptomatic.[233] Recent reports suggest that this proportion may be higher in other areas of the world, such as Brazil and other countries in Latin America.[215,218] In addition, it appears that disease severity can also be greater in countries outside Europe and the United States. For instance, community outbreaks of acute toxoplasmosis with an unusually severe clinical presentation have been recently reported from Suriname and French Guiana.[234,235] In two reports immunocompetent patients presented with severe disseminated disease, including pneumonia and hepatitis, that resulted in four deaths, including one newborn and one fetus, among 22 patients. Based on genotype analysis with microsatellite markers, "atypical" strains (i.e., not one of the three major strains seen in Europe and North America) were responsible for these outbreaks. The remarkable differences in clinical presentation of toxoplasmosis among patients from various regions of the world have significant implications when generating a differential diagnosis in travelers who become ill and are returning from highly endemic areas (e.g., Latin America).[236]

When clinical manifestations are present, toxoplasmosis most often manifests as painless cervical lymphadenopathy, but any or all lymph node groups may be enlarged. On palpation the nodes are usually discrete and nontender, rarely more than 3 cm in diameter, may vary in firmness, and do not suppurate.[237] However, the nodes may be occasionally tender or matted. Fever, malaise, night sweats, myalgias, sore throat, arthralgias, maculopapular rash, hepatosplenomegaly, or small numbers of atypical lymphocytes (<10%) may be present. The clinical picture may resemble infectious mononucleosis or CMV infection, but toxoplasmosis probably causes no more than 1% of "mononucleosis" syndromes.[238] Retroperitoneal or mesenteric lymphadenopathy may produce abdominal pain accompanied by diarrhea. Neurocognitive abnormalities appear to be common during the acute infection among immunocompetent patients.[239]

Toxoplasmic chorioretinitis as a manifestation of acute acquired infection is more common than previously recognized.[240–242] Chorioretinitis in the setting of acute acquired toxoplasmosis can occur either sporadically or in the context of an epidemic of acute toxoplasmosis.[241,243] For further discussion of this clinical entity, see "Ocular Toxoplasmosis in Immunocompetent Patients."

In most cases the clinical course of toxoplasmosis in the immunocompetent patient is benign and self-limited. Symptoms, if present, usually resolve within a few months and rarely persist beyond 12 months. Lymphadenopathy may wax and wane for months and in unusual cases for 1 year or longer. Rarely, an apparently healthy person develops clinically overt disease, for instance, fever of unknown origin or potentially fatal disseminated disease, with myocarditis, pneumonitis, hepatitis, or encephalitis. In children nephrotic syndrome has been reported in association with acute toxoplasmosis.[244] These more aggressive forms of the disease have been more commonly reported from South America.[234,235] None of the clinical presentations of acquired toxoplasmosis is distinctive; the differential diagnosis of TL includes bacterial lymphadenitis, lymphoma, infectious mononucleosis, CMV or human herpesvirus 6 (HHV-6) "mononucleosis," cat-scratch disease, sarcoidosis, tuberculosis, tularemia, metastatic carcinoma, endemic fungi (e.g., coccidioidomycosis), and leukemia. Acute acquired toxoplasmosis associated with multiorgan involvement has been reported to mimic other causes of pneumonitis, hepatitis, myocarditis, polymyositis, or fever of unknown origin in apparently immunocompetent patients.[233]

T. gondii has been estimated to cause 3% to 7% of clinically significant lymphadenopathy.[237] The major diagnostic confusion with toxoplasmic lymphadenopathy occurs with Hodgkin disease and the lymphomas. The diagnosis of recently acquired toxoplasmic lymphadenopathy is easily made serologically, but unfortunately, physicians often do not consider this diagnosis in patients with lymphadenopathy. Serologic test titers diagnostic of acute *T. gondii* infection are often obtained after histologic examination of a biopsied node has suggested the possibility of toxoplasmosis.[245]

Myocarditis as a manifestation of acute toxoplasmosis has been reported in relatively few patients.[198,246,247] It may occur clinically as an isolated disease process or as part of a variety of manifestations of disseminated infection. Manifestations include arrhythmias, pericarditis, and heart failure.[198,248]

Myositis resembling polymyositis as a manifestation of acute toxoplasmosis has also been reported infrequently.[198,249,250] Dermatomyositis has been associated with toxoplasmosis, although a cause-and-effect relationship has not been proved.[251,252]

The clinical features of toxoplasmic myocarditis and polymyositis are illustrated by a case in which both were present in the same individual.[198] A 43-year-old woman presented with cardiogenic pulmonary edema, followed by progressive sinus bradycardia and subsequent complete heart block; viral myocarditis was considered the most likely diagnosis. During the ensuing months, she developed proximal muscle weakness while being treated with corticosteroids; an endomyocardial biopsy (see Fig. 278.3C) and a quadriceps muscle biopsy (see Fig. 278.3D) revealed *T. gondii*.[198] Her symptoms improved on pyrimethamine-sulfadiazine. One year after her initial presentation with myocarditis, retinal lesions characteristic of toxoplasmic chorioretinitis were observed in her right eye. Serologic test results and follow-up were consistent with recently acquired toxoplasmosis.[198]

Several epidemiologic studies have suggested an association between infection with *T. gondii* and schizophrenia and other mental illnesses, but a definitive etiologic role of the parasite in such disorders has not been established.[123,124,253–255] Population-based studies that include following large cohorts of patients following gestation will be necessary to clarify this potential association.

Toxoplasmosis in the Immunodeficient Patient

In immunocompromised patients toxoplasmosis can present with a wide spectrum of clinical manifestations. Disseminated disease has a 100% case-fatality rate if untreated. Early diagnosis requires a high index of suspicion because routine laboratory tests aimed at detecting bacterial, viral, or fungal infections will not alert clinical laboratory personnel or the clinician to the presence of *T. gondii* as the etiologic agent. T-cell– and/or B-cell–mediated immunity defects appear to confer the highest risk for toxoplasmosis as observed in patients with hematologic malignancies (especially Hodgkin disease and other lymphomas), organ transplant recipients, or those with AIDS and those receiving immunosuppressive therapy with high doses of corticosteroids or immunomodulators, such as anti–TNF-α agents, for instance rituximab,[256] natalizumab,[257] or alemtuzumab.[258] In immunodeficient patients encephalitis, pneumonitis, and myocarditis reflect active replication and infection in the most commonly involved organs.[6] Pneumonitis is a common and underrecognized manifestation of toxoplasmosis in these patients. Fever of unknown origin may be the sole manifestation of toxoplasmosis in the early stages of the disease. Disseminated infection with multiorgan involvement is not unusual; clinical manifestations may not necessarily reflect the extent and severity of the disseminated infection. Mortality approaches 100% if the infection is not treated or is treated only late in its course. Whereas serious toxoplasmosis in these patients often reflects recrudescence of a latent infection acquired in the distant past (as observed in the setting of AIDS or HSCT), it may also result from recently acquired acute infection (as observed in solid-organ transplants through the transplanted organ) or, more rarely, through the oral route. Although clinical manifestations are similar in patients with different causes for their immunosuppression, additional considerations are provided here for the organ transplant recipient and patient with AIDS.

Toxoplasmosis in the Solid-Organ Transplant Patient

Patients with solid-organ transplants will develop toxoplasmosis most commonly as a result of acquiring *T. gondii* infection through the transplanted organ, when the allograft of a seropositive donor (D^+) is given to a seronegative recipient (R^-), resulting in a D^+/R^- mismatch (Table 278.1).[259] Toxoplasmosis can also be the result of reactivation of a previously acquired infection in the recipient, regardless of the serologic status of the donor (D^-/R^+ or D^+/R^+; see Table 278.1). Fever is often the first manifestation in transplant recipients, followed by signs referable to the brain and lungs.

TABLE 278.1 Source of Toxoplasmosis in the Organ Transplant Patient

Transplant of an Infected Organ to a Seronegative Recipient (D^+R^-)
Heart
Heart-lung
Kidney
Liver and liver/pancreas
Bone marrow (rare)
Reactivation of Latent Infection in a Seropositive Recipient (D^-R^+ and D^+R^+)
Bone marrow
Hematopoietic stem cell
Liver
Kidney (rare)

D, Donor; *R,* recipient.

Knowledge of the overall prevalence of *Toxoplasma* antibodies in a population does not accurately predict the percentage of D^+/R^- *T. gondii* mismatches. Rather, this will depend on the prevalence of *T. gondii* antibodies in the age groups of the donor and recipient populations. For example, in a given geographic area, the prevalence of antibodies in young heart donors may be 3% to 10%, whereas in the older population of individuals, who would more likely be recipients, it may be 15% to 30%. Testing for *Toxoplasma* IgG antibodies should be performed in every solid-organ transplant candidate before transplantation and on serum from every organ donor.

Since April 6, 2017, organ procurement organizations in the United States have made it mandatory to test all potential organ donors for toxoplasmosis.[260] This allows identification of those recipients at greatest risk of developing toxoplasmosis either because they were seronegative before transplantation and received an organ from a seropositive individual or because they were seropositive before transplantation and thus are at risk for reactivation of their latent (chronic) *Toxoplasma* infection. *Toxoplasma* serologies obtained in the posttransplantation period, unfortunately, are frequently not helpful, even in the presence of serious toxoplasmosis. *T. gondii* antibodies that were demonstrable before transplantation might become negative, rise, or show no change posttransplantation despite life-threatening toxoplasmosis.[261] Transfusion may further compound the difficulties encountered in serodiagnosis during the posttransplantation period.

The incidence of toxoplasmosis among various organ transplant recipients managed at 11 tertiary care hospitals in Spain between 2000 and 2009 was 0.14% (22/15,800).[262] The incidence was significantly greater in heart compared with kidney and liver recipients. The only independent risk factor identified in multivariate analysis was negative serostatus before transplant. Overall mortality was 14%, although two of the three deaths occurred in untreated patients who were diagnosed at autopsy.

At autopsy, histopathologic evidence of multiorgan involvement has been observed, most commonly of the brain, heart, and lungs but also including the eyes, liver, pancreas, adrenal, and kidney.

Heart Transplantation

In a review of infections in cardiac transplant recipients at Stanford Medical Center from 1980–96, results of serologic testing for *Toxoplasma* were available for 582 donors (35 [6%] had *T. gondii*–specific IgG antibodies) and 607 recipients (98 [16%] were positive).[263] Results of serologic testing for *Toxoplasma* were available for 575 D/R pairs; of these, 454 (79%) were D^-/R^-, 84 (14.6%) D^-/R^+, 32 (5.6%) D^+/R^-, and 5 (0.8%) D^+/R^+. Of the 32 D^+/R^- patients, 16 were receiving TMP-SMX and/or pyrimethamine prophylaxis, and none developed toxoplasmosis; however, 4 (25%) of the 16 D^+/R^- patients who were not taking either TMP-SMX or pyrimethamine developed toxoplasmosis, and all died of the infection. None of the 98 patients who were seropositive for *T. gondii*

preoperatively developed clinical evidence of reactivation of the infection. The importance of prophylaxis is further evidenced from an earlier study at Papworth Hospital in England. Fatal or severe toxoplasmosis developed in 57% (4/7) of D^+R^- mismatched heart transplant patients not receiving prophylaxis. Use of pyrimethamine, 25 mg/day for 6 weeks, reduced the transmission rate to 14% (5/37). In those patients who received pyrimethamine and were infected by the donor heart, only 1 (20%) developed symptoms of the infection in contrast to 4 of 4 (100%) who did not receive pyrimethamine prophylaxis. Subsequently, prophylaxis with TMP-SMX (80/400 mg twice daily orally for 1 year posttransplantation and when on oral prednisolone) was used in heart and lung transplant patients. Of those who were alive at 3 months post-transplantation, 28 (8.75%) were *T. gondii* mismatches; none had evidence of having acquired *Toxoplasma* infection. These investigators observed that use of prophylaxis might prolong the period before observation of seroconversion of donor-acquired infection in heart transplant patients for as long as 14 months posttransplantation.[264,265] Anti-*Toxoplasma* prophylaxis may not always work, and toxoplasmosis should still be entertained in D^+/R^- patients who present with unexplained syndromes even when there is a history of taking their prophylactic drugs.[266]

Toxoplasmosis in heart transplant recipients may simulate organ rejection. In such cases toxoplasmosis has frequently been diagnosed by endomyocardial biopsy.

It is important to recognize that many heart transplant recipients with *T. gondii* antibodies before transplantation may show increases in *T. gondii*–specific antibodies (IgG and IgM). These patients have not necessarily developed a clinical illness that can be attributed to toxoplasmosis.

Kidney Transplantation

In a review of 31 cases of toxoplasmosis in renal transplant patients the majority occurred within the first 3 months after transplantation; 3 cases occurred more than 1 year after transplantation, and 9 occurred during or immediately after a rejection episode.[267] The greatest risk was in D^+/R^- mismatches. Fever, CNS symptoms and signs, and pneumonia were the main clinical features. Chest radiographs showed bilateral pneumonia in most cases. The most common organs involved in the 15 cases diagnosed at autopsy were brain, heart, and lungs. *T. gondii* was not demonstrable in the kidneys. Whereas the overall mortality rate was 64%, 10 of 11 treated patients survived, emphasizing the importance of early diagnosis and treatment. Fatal cases of disseminated toxoplasmosis in kidney transplant recipients have been, unfortunately, unexpectedly diagnosed postmortem.[268] Acute toxoplasmosis in two recipients of renal allografts from the same donor has occurred. Chorioretinitis has been reported as the presenting manifestation of toxoplasmosis in a kidney transplant patient.[269]

Liver Transplantation

After orthotopic liver transplantation, toxoplasmosis most often results from activation of a latent infection in the transplanted allograft,[270,271] but it occurs as well from activation of a quiescent pretransplantation infection in the recipient. In most published cases clinical manifestations of toxoplasmosis appeared within the first 3 months posttransplantation. Fever was usually the first manifestation, and pneumonia, meningitis/encephalitis, and multiorgan failure were frequently observed. Retinochoroiditis requiring enucleation was observed in one patient.[272] Although it is a rare event, toxoplasmosis in this population is most often fatal.[273]

Toxoplasmosis in the Bone Marrow Transplant and Hematopoietic Stem Cell Transplant Recipient

Donor bone marrow from the patient (autologous) or a matched related or unrelated donor (allogeneic) used to be the only source of cells for BMT recipients. Stem cell transplantation is now also done using stem cells harvested from the peripheral blood (HSCT) of the patient (autologous), matched related or unrelated donor (allogeneic), matched parent (haploid), or umbilical cord blood. To keep this literature separate, HSCT and BMT are presented separately in this section. A review of 41 cases of toxoplasmosis in patients who had undergone HSCT in 15 European transplantation centers, from 1994–98, found no cases among 6787 autologous HSCTs, whereas it occurred in 0.97% of 4231 allogeneic transplants.[274] The relatively low number of cases in their large survey is likely due to the use of TMP-SMX prophylaxis after engraftment in all allogeneic BMTs in 91% of the institutions. Of the patients who develop toxoplasmosis and who had available serologies before transplantation, 94% were seropositive for *T. gondii*. Graft-versus-host disease (GVHD) had developed before toxoplasmosis in 73%. Thirty patients (73%) had not received prophylaxis for toxoplasmosis, and only 3 were receiving it at the time of disease onset. Median time of onset was day 64 (range, 4–516 days). Fever with neurologic or pulmonary symptoms was seen most commonly; myocarditis was frequently seen at autopsy. Six patients had fever without evidence of organ involvement. Twenty-two (63%) died of toxoplasmosis. It is noteworthy that of the 23 patients who received specific therapy for toxoplasmosis for greater than or equal to 6 days, 11 (48%) had a complete response, and 3 others (13%) improved. More recently, among 444 HCT patients (87% HSCT) followed prospectively in the United States between 2006 and 2011, most of whom received effective prophylaxis, no cases of toxoplasmosis were seen.[275] During a similar time period (2006–15), 3.9% of 588 transplant patients at a single center in France were diagnosed with toxoplasmosis ($n = 20$), primarily pulmonary ($n = 14$) or CNS ($n = 10$) disease, or *T. gondii* infection ($n = 3$). Effective prophylaxis was used less routinely (22% of cases, 70% of control subjects).[276] Toxoplasmosis was also seen in 2.1% (4/187) of Italian pediatric HSCT patients.[277]

In 106 *T. gondii*–seropositive adult recipients of HSCTs the incidence of reactivation of toxoplasmosis in the first 6 months after transplantation was prospectively studied.[278] *Toxoplasma* serologies had been obtained pretransplantation. The incidence of reactivation within 6 months after transplantation, as established by a positive PCR test result in peripheral blood, was observed in 16 of the 106 patients (16%). Of the 16 patients with positive PCR test results, 6 (38%) developed disease (toxoplasmosis). Thus PCR may be useful in monitoring at-risk patients (i.e., seropositive patients identified in the pretransplantation period) (see "Diagnosis").

Survival in this setting is highest in patients with ocular and/or isolated cerebral toxoplasmosis, primarily when treatment is begun as soon as the diagnosis is suspected.[96,274] Survival in the presence of disseminated toxoplasmosis is rare in these HSCT patients.[96,274] Although reactivation of latent *Toxoplasma* infection in allogeneic HSCT recipients most often occurs in the first 6 months posttransplantation (the majority occur in the first 30–90 days), late reactivation has been observed and must be considered in patients in whom late-onset (beyond 6 months posttransplantation) GVHD occurs.[278,279]

The incidence of toxoplasmosis in BMT has been reported to range from 0.3% to 5% and is influenced by the prevalence of pretransplantation antibodies and whether toxoplasmosis prophylaxis was used. Major risk factors for toxoplasmosis included the presence of pretransplantation *T. gondii* antibodies in recipients and the occurrence of GVHD. In a review of 110 published cases of toxoplasmosis after BMT,[96] 96% occurred after allogeneic BMT. Onset of infection posttransplantation occurred on days 1 to 30 in 13%, on days 31 to 100 in 64%, and on days past 100 in 23%. The infection occurred primarily in recipients who were seropositive pretransplantation (88%), meaning infection was more often reactivation in the recipient, not transplanted from the donor. The diagnosis was made antemortem in only 47% of the cases. Overall mortality was 80% (median, 87 days posttransplantation), and in 66% it was attributed to toxoplasmosis (median, 74 days posttransplantation). Patients with isolated cerebral involvement had a better outcome (58% survival) than patients with disseminated toxoplasmosis (20% survival); underlying disease was the only factor associated with clinical presentation, with acute leukemia being more common in patients with disseminated disease.

TMP-SMX prophylaxis, primarily used by transplantation teams to prevent *Pneumocystis* pneumonia, has been successful in prevention of toxoplasmosis. In HSCT patients, this is usually begun after engraftment because of the potential of the drug combination for bone marrow suppression. The delay in instituting prophylaxis likely results in many more cases of toxoplasmosis than would be expected to occur if adequate prophylaxis was begun early after transplantation. This problem highlights the importance of PCR monitoring of at-risk patients and identifying additional drugs for prophylaxis in these patients.[280,281]

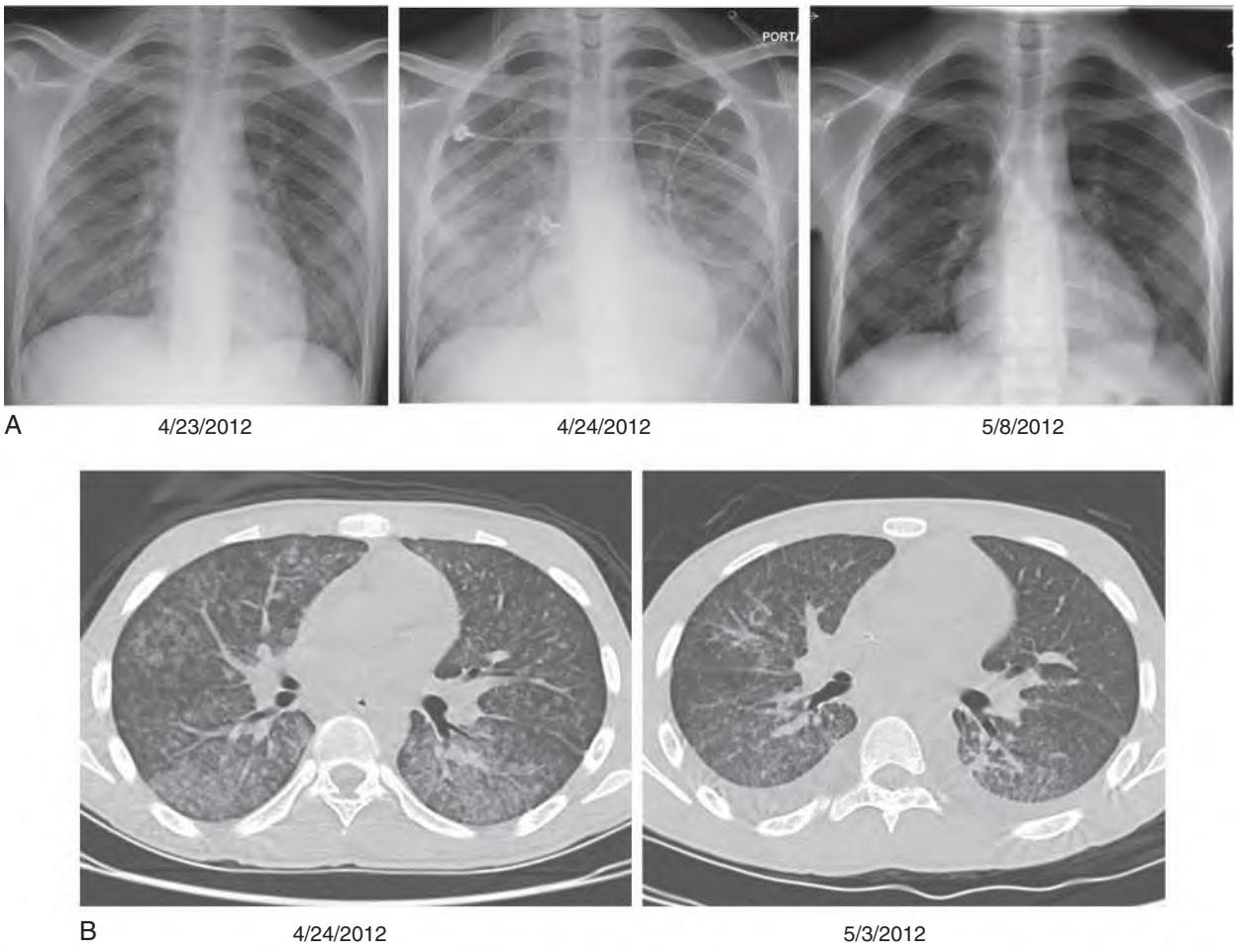

FIG. 278.4 Pulmonary toxoplasmosis. Serial chest radiographs (A) and computed tomography scans of the chest (B) of a patient who had received a haplocord transplant as treatment for myelodysplastic syndrome complicating aplastic anemia and subsequently presented with pulmonary symptoms. Pulmonary toxoplasmosis was diagnosed by polymerase chain reaction (PCR) of a bronchoalveolar lavage sample obtained April 24, 2012. The patient also had a positive PCR for toxoplasmosis in blood and cerebrospinal fluid. The patient responded to treatment with intravenous trimethoprim-sulfamethoxazole and subsequently oral pyrimethamine-sulfadiazine-leucovorin, with resolution of pneumonia and conversion of PCR to negative. *(Courtesy Dr. Richard Childs, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.)*

Toxoplasmosis in BMT patients frequently involves the lung, usually in the setting of multiorgan disease, with a high associated mortality (>90%) (Fig. 278.4). Most patients die within 7 days of onset of pulmonary symptoms and often suffer acute respiratory distress syndrome. In a review of 25 cases of pulmonary toxoplasmosis in BMT, onset of symptoms referable to the lungs occurred from 7 days to 1 year post-BMT; the majority occurred in the first 6 weeks.[282] Fever may be the first sign; if pulmonary infiltrates are observed, especially in the setting of rapid deterioration, immediate attempts at diagnosis must be made and empirical treatment begun. On CT scan of the lungs, most patients present with bilateral ground-glass opacities.[283] In HSCT patients with this radiologic pattern, the differential diagnosis should include *Pneumocystis* pneumonia (PCP), atypical pneumonia (e.g., *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*), viral pneumonia (e.g., CMV), strongyloidiasis, diffuse alveolar hemorrhage, pulmonary edema, and drug hypersensitivity. However, toxoplasmosis is often overlooked by those generating differential diagnoses in this setting, including radiologists, cancer and organ transplant providers, and infectious diseases specialists. Undoubtedly, the high mortality in many instances has been due to lack of early diagnosis and treatment. The organism can be observed on microscopic examination of BAL material. PCR on material obtained at BAL is the diagnostic procedure of choice.[282] PCR also can be performed on blood, serum, CSF, and bone marrow aspirates.

Ocular toxoplasmosis has been reported after allogeneic and autologous BMT and HSCT. In some cases the ocular disease was due to reactivation of a previously observed toxoplasmic chorioretinitis, whereas in most it has been associated with disseminated infection. Definitive diagnosis has been by direct observation of the parasite in histopathology sections, culture of tissue samples, or by PCR on vitreous fluid.

Toxoplasmosis in the AIDS Patient

Clinical manifestations of toxoplasmosis in AIDS patients commonly reflect encephalitis (i.e., TE), or infection of the lung (pneumonitis), and the eye (chorioretinitis).[284] Toxoplasmosis with multiorgan involvement manifesting with acute respiratory failure and hemodynamic abnormalities similar to septic shock has been reported, although septic shock has not been definitely proved to be due to *T. gondii*.[285] TE is the most common presentation of toxoplasmosis in AIDS patients and is a frequent cause of focal CNS lesions in these patients.[100] A wide range of clinical findings, including altered mental state, seizures, weakness, cranial nerve disturbances, sensory abnormalities, cerebellar signs, meningismus, movement disorders, and neuropsychiatric manifestations are seen in TE. The characteristic presentation usually has a subacute onset with focal neurologic abnormalities in 58% to 89% of patients. However, in 15% to 25% of cases the clinical presentation may be more abrupt, with seizures or cerebral hemorrhage. Most common, hemiparesis or abnormalities of speech, or both, are the major initial manifestations. Brainstem involvement often produces cranial nerve lesions, and many patients exhibit cerebral dysfunction with disorientation, altered mental state, lethargy, and coma. Less common, parkinsonism, focal dystonia,

rubral tremor, hemichorea-hemiballismus, panhypopituitarism, diabetes insipidus, or the syndrome of inappropriate antidiuretic hormone secretion may dominate the clinical picture. In some patients neuropsychiatric symptoms, such as paranoid psychosis, dementia, anxiety, and agitation, may be the major manifestations.

Diffuse TE[208] has been reported in relatively few AIDS patients; its actual incidence is unknown. This form of TE may manifest acutely and can be rapidly fatal; generalized cerebral dysfunction without focal signs is the most common manifestation, and CT scans may be within normal limits or reveal cerebral atrophy.

Spinal cord involvement by *T. gondii* in AIDS patients manifests as motor or sensory disturbances of single or multiple limbs, bladder or bowel dysfunctions (or both), and local pain. Patients may present with a clinical syndrome resembling a spinal cord tumor. Reports of cervical myelopathy,[286] thoracic myelopathy,[287] and conus medullaris syndrome[288] have been published.

Pulmonary disease caused by toxoplasmosis has been reported in patients with AIDS, and the diagnosis may be made by demonstration of the parasite in BAL fluid.[289] In France, before ART and routine use of prophylaxis, the prevalence of pulmonary toxoplasmosis in patients dually infected with HIV and *T. gondii* was estimated to be approximately 5%.[290] Pulmonary toxoplasmosis occurs mainly in patients with advanced AIDS (mean CD4 count, 40 cells/mm^3 ± 75 standard deviation) and primarily presents as a prolonged febrile illness with cough and dyspnea,[289] which may be clinically indistinguishable from *Pneumocystis* pneumonia. Mortality, even when treated appropriately, may be as high as 35%. Extrapulmonary disease may be present in about 50% of cases with toxoplasmic pneumonitis.[285] Often, pulmonary toxoplasmosis is not associated with TE; however, TE may develop after successful treatment of pulmonary toxoplasmosis when therapy is discontinued. The differential diagnosis of toxoplasmic pneumonitis in AIDS patients includes *Pneumocystis* pneumonia, viral pneumonia (e.g., caused by CMV or community-acquired respiratory viruses), pneumonia caused by atypical pathogens (e.g., *Chlamydia* spp., *Mycoplasma pneumoniae*), and infection with *M. tuberculosis, Cryptococcus neoformans*, *Coccidioides* spp., and *Histoplasma capsulatum*.

Toxoplasmic chorioretinitis is seen relatively infrequently in AIDS patients[291]; it commonly manifests with ocular pain and loss of visual acuity (Fig. 278.5). Funduscopic examination usually demonstrates necrotizing lesions that may be multifocal or bilateral.[291] Overlying vitreal inflammation is often present and may be extensive. The optic nerve may be involved in as many as 10% of cases. Toxoplasmic chorioretinitis in AIDS patients has been associated with concurrent TE in up to 63% of patients. The differential diagnosis of toxoplasmic chorioretinitis in AIDS patients includes CMV retinitis, syphilis, herpes simplex, varicella zoster, and candidiasis. The diagnosis relies primarily on clinical findings and the response to anti–*T. gondii* therapy, although definitive diagnosis may be made by demonstration of the organism in retinal biopsies,[291] isolation of the parasite from vitreous aspirates,[292] or amplification of the parasite DNA by PCR.[212]

GI involvement may result in abdominal pain, ascites (resulting from involvement of the stomach, peritoneum, or pancreas), or diarrhea. Acute hepatic failure caused by *T. gondii* has been reported,[227] as has musculoskeletal involvement.[220]

Although the incidence of toxoplasmosis in HIV-infected patients began to decrease after the broad use of TMP-SMX for PCP prophylaxis, the incidence decreased dramatically as a result of the immune reconstitution associated with ART regimens. Approximately fourfold decreases in both incidence and death have been reported after the wide availability of ART regimens.[101,103,293] Estimated hospitalization rates for HIV-related toxoplasmosis similarly decreased from a peak of ≈10,500 to ≈3,000 in the early 2000s but subsequently stabilized at that level.[102] Yet despite this, TE remains one of the most common HIV-related neurologic disorders, accounting for 26% of such cases in one recent study, with a 1-year estimated survival of 77%.[294] In contrast to mycobacterial and cryptococcal infections, immune reconstitution inflammatory syndrome (IRIS) after the initiation of ART has been only rarely reported in association with toxoplasmosis.[295–298] A recent retrospective review of patients followed at six Dutch hospitals identified paradoxical IRIS at a median of 49 days after starting combination antiretroviral therapy (cART) in 3.5% of 143 patients with previously diagnosed TE, and unmasking IRIS in 0.36% of 2228 patients with no prior diagnosis at a median of 35 days after starting cART.[299] In patients with CD4 counts that are sustained to levels greater than 200 cells/mm^3 for 3 to 6 months while receiving ART, primary prophylaxis and chronic maintenance regimens can be safely discontinued, although rare cases of disease can occur even in patients with well-controlled HIV infection.[300–303] Primary

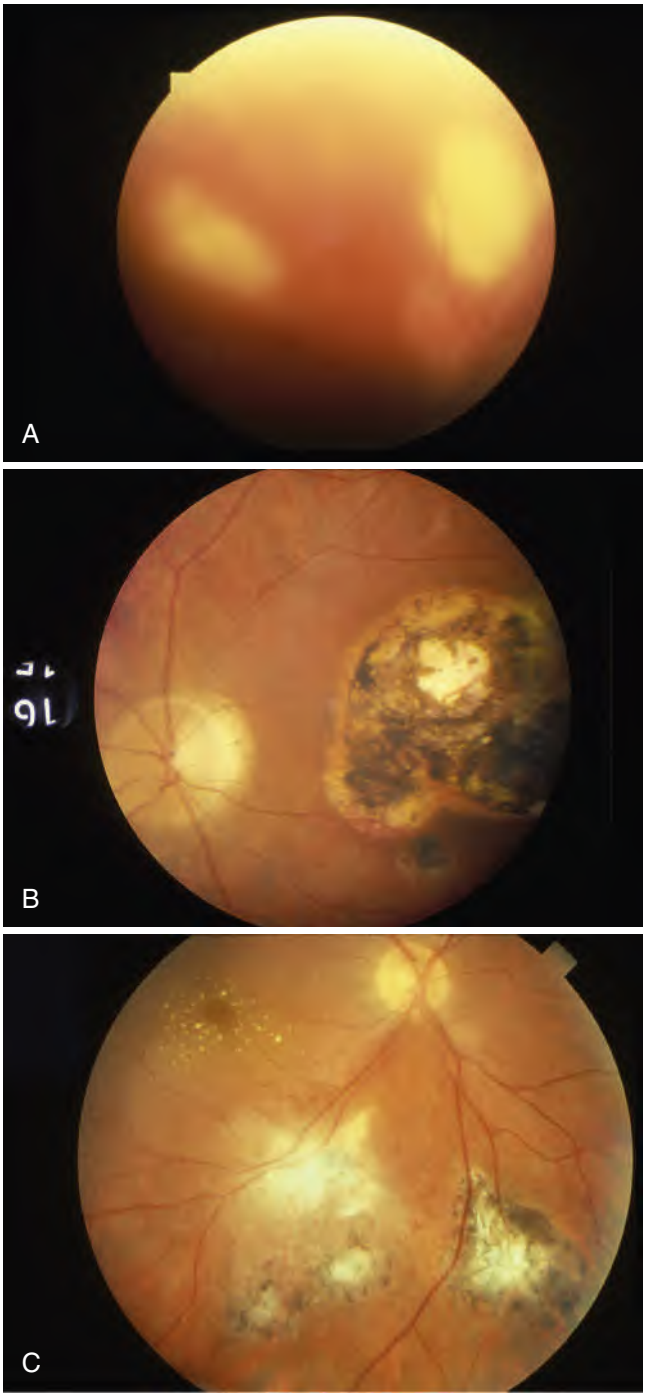

FIG. 278.5 Toxoplasmic chorioretinitis. (A) Active chorioretinitis with two lesions and vitreous haze in a human immunodeficiency virus–infected patient. (B) A large inactive macular lesion typical of congenital disease. (C) Active chorioretinitis in an immunocompetent patient from an endemic region in Brazil. There is an inactive lesion on the right. Note the macular star, which is due to an exudate around the macula. *(Courtesy Dr. Robert Nussenblatt, National Eye Institute, National Institutes of Health, Bethesda, MD; Dr. Claudio Silveira, Erechim, Brazil; and Dr. Rubens Belfort, São Paulo, Brazil.)*

prophylaxis can also be discontinued in those with CD4 counts of 100 to 200 cells/μL in the setting of controlled viremia (below detection limits for at least 3–6 months).[304]

There are no data on whether the strain of *T. gondii* affects the clinical outcome of infection in AIDS patients, and there are conflicting reports on whether a particular strain is more likely to cause infection in such patients.[18,305]

Ocular Toxoplasmosis in Immunocompetent Patients

T. gondii is one of the most frequently identified etiologies of uveitis and the most commonly identified pathogen to infect the retina of otherwise immunocompetent individuals.[306] It accounted for 8.4% of cases of uveitis seen in a clinic in San Francisco over a 24-year period.[307] Toxoplasmosis is responsible for more than 85% of posterior uveitis cases in southern Brazil, where in one study 9.5% of 21 seroconverters and 8.3% of 131 seropositive patients without ocular involvement developed typical lesions during 7 years of follow-up.[308–310] Chorioretinal lesions may result from congenital or postnatally acquired infection. In both these situations lesions may occur during the acute or latent (chronic) stage of the infection.[241,311] Recurrences are frequent, occurring in 79% of patients followed for more than 5 years in one study, with a median time to recurrence of 2 years; they tend to occur in clusters, that is, immediately after an episode rather than randomly, are more common in patients older than 40 years, are more common in the eye originally involved, and may be more common after cataract extraction.[312–314]

It is frequently difficult to determine whether the original infection was congenital or acquired in patients who suffer recurrences of chorioretinitis.[242] Although previously it was thought that less than 1% of cases were acute infections, the remainder being reactivations, recent evidence indicates that 11.7% of US ocular infections are acute.[315] Patients who present with chorioretinitis as a late sequela of the infection acquired in utero tend to have more severe disease and are more frequently in the second and third decades of life (it is rare after the age of 40 years); bilateral disease, old retinal scars, and involvement of the macula are hallmarks of the retinal disease in these cases, as are recurrences (see Fig. 278.5).[9] By contrast, patients who present with toxoplasmic chorioretinitis in the setting of acute toxoplasmosis are more often between the fourth and sixth decades of life, most often have unilateral involvement, and have eye lesions that usually spare the macula and do not present with associated old scars.[241,242]

Whereas acquired *T. gondii* infection in otherwise healthy adults is most often subclinical, toxoplasmic chorioretinitis in these individuals may result in retinal detachment, complete or partial loss of vision, or glaucoma, and it may necessitate enucleation.[9,316–318] Acute chorioretinitis may produce symptoms of blurred vision, scotoma, pain, photophobia, and epiphora. Impairment or a loss of central vision occurs when the macula is involved. As inflammation resolves, vision improves, frequently without complete recovery of visual acuity, caused in large part by macular scar formation.[307] In a cohort of 230 patients recently followed prospectively in Brazil for a mean of nearly 3 years, 45% experienced recurrent episodes, 4% developed a lesion in the previously unaffected eye, and 53% had decreased visual acuity (<20/30) at the end of follow-up.[317] In most cases toxoplasmic chorioretinitis is diagnosed by ophthalmologic examination, and empirical therapy directed against the organism is often instituted based on clinical findings and serologic test results. Typical features of toxoplasmic chorioretinitis include intensely white focal lesions with an overlying, intense, vitreous inflammatory reaction (see Fig. 278.5). After acute infection, lesions may present with focal retinal whitening only; such patients are at higher risk for developing chorioretinitis than patients with no lesions.[319] Focal necrotizing retinitis initially appears in the fundus as a yellowish-white, elevated cotton patch with indistinct margins, usually on the posterior pole. The lesions are often in small clusters, and individual lesions in the cluster may be of varied ages. With healing, the lesions pale, atrophy, and develop black pigment (see Fig. 278.5). There can also be an associated, secondary iridocyclitis and increased intraocular pressure.[316] The classic "headlight in the fog" appearance is due to the presence of active retinal lesions with severe vitreous inflammatory reaction. The choroid is secondarily inflamed. Recurrent lesions tend to occur at the borders of chorioretinal scars, and scars are often found in clusters. Panuveitis may accompany chorioretinitis, but isolated anterior uveitis has never been proved to occur.

Although the morphology of the lesions of acute toxoplasmic chorioretinitis in the setting of postnatally acquired disease may be indistinguishable from those observed in patients who suffer acute eye disease in later life caused by a congenitally acquired infection, it is important to attempt to establish which type of the infection (postnatally acquired or congenital) is occurring in a given patient.[241] It appears that the congenitally acquired disease has a more guarded prognosis. From the public health perspective, it is important epidemiologically to establish whether the patient has acute acquired infection, to initiate efforts to identify the possible source of *T. gondii* infection, and to determine whether other individuals who may be at high risk for developing severe, life-threatening disease (i.e., fetuses of serologically negative pregnant women or immunodeficient individuals) shared the same exposure as the individual with acute acquired toxoplasmic chorioretinitis. Serologic tests have been useful in establishing whether such patients have been infected recently.[240,241] In patients with chorioretinitis and IgG antibodies, additional serologic tests should be performed to determine whether the patient's infection is recently acquired.[241]

T. gondii chorioretinitis may resemble the posterior uveitis of tuberculosis, syphilis, leprosy, or presumed ocular histoplasmosis syndrome.

Atypical clinical and serologic manifestations of toxoplasmic chorioretinitis have been reported most commonly in elderly and in immunodeficient individuals.[212,320] Patients are considered to have atypical-appearing lesions when one or more of the following features are present: multiple foci of active retinitis, acute retinal necrosis syndrome (vitritis, peripheral retinitis, retinal vasculitis), significant intraretinal hemorrhage, an absence of ophthalmoscopically visible chorioretinal scarring. In patients with atypical lesions or an inadequate clinical response to anti-*Toxoplasma* therapy or in whom other diagnostic procedures have not proved helpful, obtaining vitreous or aqueous fluid (in some cases indicated for therapeutic reasons as well) for PCR should be considered early in the workup (see "Diagnosis").[213] In the future it may be possible to routinely determine which strain of the parasite is responsible for the infection and make a more accurate prognosis. Preliminary indications are that strain type may play an important part in determining the course of the infection,[218,219] and methods to distinguish strain type by looking for strain-specific antibodies using polymorphic peptides have shown promise.[23,87,321]

Toxoplasmosis During Pregnancy

As in other immunocompetent individuals, acute *Toxoplasma* infection is asymptomatic in the majority of pregnant women. Based on newly developed serologic assays that can detect antibodies to sporozoites,[60] the majority of women in one US study appeared to have been initially infected with oocysts, and a high proportion were unable to identify the potential source of such infection.[322] Given these factors, women should be universally screened by serology to maximize diagnosis of toxoplasmosis during pregnancy. The most commonly recognized clinical manifestation of recent infection is regional lymphadenopathy. The primary concern is transmission of infection to the fetus. The risk to the fetus does not correlate with whether the infection in the mother was symptomatic or asymptomatic during gestation. Transmission to the fetus has been limited almost solely to those women who acquire the infection during gestation. Otherwise healthy women with prior *Toxoplasma* infection are protected from transmitting the infection to their fetuses. Rare exceptions to this dictum have been observed. In immunocompetent women infected with *T. gondii* shortly before conception, transmission to the fetus has occurred; in these very rare instances, the acute infection was acquired within 3 months of conception.[323–325] Transmission to the fetus has been rarely recognized as a consequence of reactivation of latent *T. gondii* infection in immunocompromised women infected with *T. gondii* before conception (chronic infection) (e.g., pregnant women coinfected with HIV and *T. gondii*,[326] patients with systemic lupus erythematosus who are being treated with corticosteroids). In addition, reinfection with a second, more aggressive strain of *T. gondii* of otherwise healthy pregnant women who are already

chronically infected with the parasite has been proposed and documented as a possible mechanism of transmission to the fetus.[128,323]

Congenital Toxoplasmosis

Congenital infection may present in one of five forms: (1) ultrasound abnormalities consistent with toxoplasmosis or positive amniotic fluid PCR test results in the fetus; (2) neonatal disease; (3) disease (mild or severe) occurring in the first months of life; (4) sequelae or relapse of a previously undiagnosed infection during infancy, childhood, or adolescence; and (5) subclinical infection.

Data accumulated from prospective studies indicate that the incidence and severity of congenital toxoplasmosis vary with the trimester during which the infection was acquired by the mother.[9] Moreover, there is an inverse relationship between the frequency of transmission and the severity of disease. Infants born of mothers who acquire their infection in the first and second trimester more frequently show severe congenital toxoplasmosis.[327] In contrast, the majority of children born of women who acquire their infection during the third trimester are born with the subclinical form of the infection. However, if left untreated, as many as 85% of these latter children develop signs and symptoms of the disease, in most cases chorioretinitis or delays in development.[328,329] Although the majority of neonates born to mothers infected later in gestation have subclinical infection, recent reports suggest that when infected with a more virulent or atypical strain, severe congenital disease may occur despite having acquired their infection late in gestation.[330]

Infection acquired in the first trimester by women who were not treated with anti–*T. gondii* drugs resulted in congenital infection in 25% of cases in one report.[327] For second- and third-trimester infections, the incidences of fetal infection were 54% and 65%, respectively.[327] The overall rate of vertical transmission in this study of untreated women was 50%.[327] Early treatment of the mother with spiramycin appears to reduce the incidence of congenital infection by about 60%.[327,331–334] Maternal infection acquired around the time of conception and within the first 2 weeks of gestation and treated with spiramycin usually does not result in transmission.[333] Because of the high transmission rates observed in the late second trimester and during the third trimester, it is recommended that pyrimethamine-sulfadiazine be used in patients in whom acute infection is highly suspected or confirmed as having occurred at 14 weeks of gestation or later.[334a,334b]

Frequency of transmission to the fetus and severity of disease in the offspring appear to be significantly affected by drug treatment with spiramycin and pyrimethamine-sulfadiazine as well. In cohorts where most of the mothers have been treated during gestation, transmission rates were low in the first trimester. (i.e., 6% [95% confidence interval [CI], 3% to 9%)] at 13 weeks) and increased as expected with advancing gestational age (i.e., 40% [95% CI, 33% to 47%] at 26 weeks and 72% [95% CI, 60% to 81%] at 36 weeks).[335] The overall rate of vertical transmission in this study of treated women was 29% (95% CI, 25% to 33%).[335] Clinical signs in the infected infant were more likely observed in offspring of women whose infection was acquired early in gestation. Depending on when during gestation the mother acquired her infection, the risk for severity of clinical manifestations in an infected fetus was 61% (95% CI, 34% to 85%) at 13 weeks, 25% (95% CI, 18% to 33%) at 26 weeks, and 9% (95% CI, 4% to 17%) at 36 weeks.[335] A recent study reported the lowest rates of overall transmission (4.8%) and clinical manifestations in the newborn (1.6%) published to date.[336] In this cohort pregnant women with *T. gondii* infection acquired during gestation received spiramycin until the 16th week of gestation, followed by at least 4 weeks of a pyrimethamine-sulfadiazine–folinic acid combination independent of the infection status of the fetus. If fetal infection was suspected (e.g., ultrasound abnormalities suggestive of congenital infection are observed) or confirmed (e.g., positive amniotic fluid PCR test), combination treatment was continued until delivery. In their cohort their rates of transmission for the first, second, and third trimester were 1.3%, 10.6%, and 21.7%, respectively. Another study reported that prenatal treatment with spiramycin and/or pyrimethamine-sulfadiazine resulted in a significantly reduced risk of severe neurologic sequelae or death in the infected offspring.[337] Of note, recently severe congenital toxoplasmosis has only been reported from countries such as the United States and Brazil, where universal screening and treatment during gestation have not been implemented.[338,339]

In addition to gestational age and treatment, transmission rates and severity of congenital disease are also likely to be affected by the strain of the parasite,[340,341] infecting form of the parasite (cyst vs. oocyst), parasite load,[342,343] and immune status and genetics of the host.

Clinical manifestations of congenital toxoplasmosis vary. Most signs and clinical presentations are nonspecific and may mimic disease caused by organisms such as herpes simplex virus (HSV), CMV, Zika virus, and rubella virus. Signs include chorioretinitis, strabismus, blindness, epilepsy, psychomotor or mental retardation, anemia, jaundice, rash, petechiae resulting from thrombocytopenia, encephalitis, pneumonitis, microcephaly, intracranial calcification, hydrocephalus, diarrhea, hypothermia, and nonspecific illness.[9] There may be no sequelae, or sequelae may develop or be evident at various times after birth.

A detailed examination by an experienced clinician may be necessary to detect signs of the infection.[9] In one prospective study[344] 210 congenitally infected infants were identified: 2 patients (0.9%) died, 21 (10.9%) had severe disease, 71 (33.8%) were mildly afflicted, and 116 (54.4%) were without signs of the infection. More intensive examination of the latter 116 infants revealed abnormalities in 39; abnormal CSF was detected in 22 infants, chorioretinitis was seen in 17, and intracranial calcifications were found in 10. Premature infants often suffer CNS disease and ocular disease in the first 3 months of life. Full-term infants frequently develop a milder disease manifested by hepatosplenomegaly and lymphadenopathy that usually appear in the first 2 months of life. In these infants, disease reflecting damage to the CNS may occur later, and eye disease may occur months to years after birth.

Most untreated infants with subclinical infection at birth subsequently develop signs or symptoms of congenital toxoplasmosis, including chorioretinitis, which may lead to blindness, neurologic manifestations such as seizures, and sensorineural hearing loss.[329,345] Prospective observational studies suggest that early initiation of specific therapy (prenatally and postnatally) in infants with congenital infection but without clinical signs will markedly reduce untoward sequelae.[346,347] In one cohort, among children who were diagnosed with toxoplasmic chorioretinitis only after their first year of life (thus who were not treated during their first year of life), new chorioretinal lesions were detected in more than 70%,[348] whereas among 108 congenitally infected children treated during their first year of life, only 31% developed at least one new chorioretinal lesion. Thirteen percent had occurrences when they were 10 years or older, indicating that long-term follow-up is important.[346,349] Similar results were seen in a prospective French study of 477 treated patients followed for up to 22 years.[350] It appears that, when treated, initially asymptomatic congenital toxoplasmosis has an overall good prognosis.[346]

Uncommonly, latent *T. gondii* infection may reactivate in HIV-infected women and result in congenital transmission of the parasite. Congenital toxoplasmosis appears to occur more frequently in the offspring of women infected with both HIV and *T. gondii* than in those of women who are infected with *T. gondii* but not with HIV.[351] Infants with congenital toxoplasmosis born to HIV-infected mothers are also infected with HIV, suggesting that factors predisposing to the vertical transmission of HIV also favor the transmission of *T. gondii*, or vice versa. Congenital toxoplasmosis in the HIV-infected infant appears to run a more rapid course than that in the non–HIV-infected infant, with the development of failure to thrive, fever, hepatosplenomegaly, chorioretinitis, and seizures. Most children have multiorgan involvement, including CNS, cardiac, and pulmonary disease.

It has been recently reported that *T. gondii* causes more severe ocular disease in congenitally infected children in Brazil when compared with those in Europe.[216] As stated earlier, this may be explained by the fact that pregnant women in Brazil are not routinely screened and treated for toxoplasmosis during gestation and possibly that more virulent genotypes of the parasite predominate in Brazil but are rarely found in Europe.

Congenital toxoplasmosis must be differentiated from rubella virus, CMV, Zika virus, HSV, HHV-6, parvovirus B19, and lymphocytic choriomeningitis virus infections; syphilis, listeriosis, and other bacterial infections; other infectious encephalopathies; erythroblastosis fetalis; and sepsis. HSV, CMV, Zika virus, rubella virus, and syphilis may cause

chorioretinitis; both CMV and rubella have been associated with hydrocephalus, microcephaly, and cerebral calcification. Zika virus particularly has been associated with microcephaly.[352] A markedly elevated CSF protein concentration is a hallmark of congenital toxoplasmosis.

T. gondii infection acquired during pregnancy has been implicated in spontaneous abortion, stillbirth, and premature births. On rare occasions *T. gondii* has been isolated from the abortuses of women with chronic infection, but the frequency of *T. gondii* infection as a cause of abortion is unknown and controversial.

As with ocular disease the strain of *T. gondii* may have an impact on the outcome of congenital infection. Studies in Europe have revealed conflicting data on whether type I strains, in particular, might be more likely to be responsible for congenital infection and/or serious disease.[19,20,353] Non–type II strains were associated with prematurity and severity of disease at birth in a study from the United States that used serologic tests as a method to determine the strain type.[354] A recent meta-analysis found that type I and atypical strains were associated with clinical complications.[340] As more accurate and sensitive tests for determining the genotype of an infecting strain are developed, this picture could change significantly.

DIAGNOSIS

When considering toxoplasmosis in the differential diagnosis of a patient's illness, emphasis should not be placed on whether the patient has been exposed to cats. Transmission of oocysts virtually always occurs without the knowledge of the patient and may be unrelated to direct exposure to a cat (e.g., transmission by contaminated vegetables, other foods, or water). Patients with an indoor cat that is fed only cooked food are not at risk of acquiring the infection from that cat. Serologic investigation of a cat to establish whether it is a potential source of the infection is useless and should be discouraged; the prevalence of *T. gondii* antibodies among cats in a given locale is usually similar to their prevalence in humans. Seropositivity does not predict shedding of oocysts. In several epidemiologic studies in Europe and the United States, acute *Toxoplasma* infection has been documented in patients without symptoms and without known epidemiologic risk factors for acute infection.[355,356]

Because the clinical manifestations of *T. gondii* infection may be protean and nonspecific, toxoplasmosis must be carefully considered in the differential diagnosis of a large variety of clinical presentations. The correct diagnostic tests must be performed and appropriately interpreted in light of the patient's clinical presentation. The usefulness of a given diagnostic method may differ considerably with the clinical category, which can be toxoplasmosis in the immunocompetent or immunodeficient patient, ocular toxoplasmosis, toxoplasmosis in pregnancy, or congenital toxoplasmosis.[357]

Acute infection is diagnosed by characteristic serologic test results; demonstration of tachyzoites in tissue or body fluids; amplification of *T. gondii* DNA or isolation of the parasite in body fluids; the demonstration of a characteristic lymph node histologic appearance; or demonstration of *T. gondii* tissue cysts in the placenta, fetus, or neonate.[9] Rarely, asymptomatic patients with latent infection have recurrent parasitemia.[358] Amplification of parasite DNA or isolation of *T. gondii* from the tissues of older children or adults may only reflect the presence of tissue cysts. Finding numerous tissue cysts in tissue sections, especially associated with inflammation, suggests but does not prove the presence of active infection.

Serologic Tests for Demonstration of Antibody

The use of serologic tests for the demonstration of specific antibody to *T. gondii* tachyzoites or antigens is the primary method of diagnosis. A large number of tests have been described, some of which are available only in highly specialized laboratories. Different serologic tests often measure different antibodies that possess unique patterns of rise and fall with the time after infection.[357] However, initial serologic testing can be accomplished by simultaneously requesting IgG and IgM antibody tests. This task can be easily achieved by hospital-based, commercial, or nonreference laboratories. Only positive results in IgM antibody tests need to be sent for confirmatory testing to reference laboratories (see later).[359]

There is no single serologic test that can be used to support the diagnosis of acute or chronic infection by *T. gondii*. In most cases a battery of tests is required to enable the distinction between acute and chronic infection. Which particular combination of tests is used depends on the specific clinical category of the patient (i.e., pregnant vs. immunodeficient patient; see "Clinical Manifestations"), the interval between acquisition of infection and sampling of sera,[245] and the question posed by the practitioner. The clinician must be familiar with these problems and consult reference laboratories if the need arises. A panel of tests consisting of the Sabin-Feldman dye test (DT; detecting IgG), the IgM-, IgA-, and IgE–enzyme-linked immunosorbent assays (ELISAs), IgM immunosorbent agglutination assay (IgM-ISAGA) (bioMérieux; Marcy-l'Étoile, France), differential agglutination test (measures IgG antibody and is also known as the AC/HS test), and the IgG avidity test is used successfully by the PAMF-TSL to determine whether serologic test results are more likely consistent with infection acquired in the recent or more distant past.[198,241,245,360–362] It is also important to note that in 2011 a test was reported that showed great promise for discriminating between an infection derived from ingestion of oocysts versus tissue cysts.[60] It relies on detection of antibodies to a protein that is abundantly expressed in sporozoites but not in tachyzoites or bradyzoites, and it has the potential to address both the epidemiologic origins of infection in different cohorts/regions and could also allow a determination of whether disease initiated by one or other means leads to different clinical outcomes. What follows concerns only the generic detection of a *Toxoplasma* "infection," that is, tachyzoites, without regard to the original source of that infection, but such refinement is something anxiously anticipated for the near future.

Immunoglobulin G Antibodies

The most widely used tests for the measurement of IgG antibody are the Sabin-Feldman DT,[363] ELISA,[364,365] the indirect fluorescent antibody (IFA) test,[366] and the modified direct agglutination test.[367] In these tests IgG antibodies usually appear within 1 to 2 weeks of acquisition of the infection, peak within 1 to 2 months, fall at variable rates, and usually persist for life at relatively low titers.

Reports have shown that detecting antibodies to strain-specific peptides can reveal the genotype of the infecting strain; although this approach has been helpful in epidemiologic studies, it has not yet been developed into a reliable, clinically useful diagnostic method.[321,354]

Sabin-Feldman Dye Test

The Sabin-Feldman DT is the reference serologic test against which other methods have been evaluated.[363] It is a sensitive and specific neutralization test. It measures primarily IgG antibodies that usually appear 1 to 2 weeks after the initiation of infection, reach peak titers in 6 to 8 weeks, and then gradually decline over 6 to 12 months.[9] Titers, usually at low levels, probably persist for life. This test is available in only a few reference laboratories, primarily because live organisms are required. A negative Sabin-Feldman DT practically rules out prior exposure to *T. gondii*, except in patients who have been infected very recently (e.g., within 1–2 weeks of exposure), are significantly immunocompromised (e.g., allogeneic BMT patients), or who have a primary immunodeficiency (e.g., congenital agammaglobulinemia).

Recently, patients with established *T. gondii* infection have been documented to have lost their *Toxoplasma* IgG (and evidence of T-cell mediated immunity as well), likely indicating that lifelong persistence may not be universal in humans.[368] Early in infection (e.g., within 4 weeks of infection), patients may be negative in IgG-ELISA, agglutination, or IFA kits but positive in the DT. However, although rare, cases of documented TE and chorioretinitis have been reported in DT-negative patients.

Indirect Fluorescent Antibody Test

The IFA test appears to measure the same antibodies as the DT, and its titers tend to parallel DT titers.[9] False-positive results may occur with sera that contain antinuclear antibodies,[369] and false-negative results may occur in sera with low IgG antibody titers. Use of the *Toxoplasma* IFA test should be discouraged because of the relative high frequency of false-negative and false-positive results.

Agglutination and AC/HS Tests

The agglutination test using formalin-preserved whole tachyzoites is available commercially (bioMérieux) and detects IgG antibody. The test is very sensitive to IgM antibody, and "natural" IgM antibody causes nonspecific agglutination in sera that yield negative results when tested in the DT and the IFA test. This problem is avoided by including 2-mercaptoethanol in the test. The method is accurate, simple to perform, inexpensive, and excellent for screening purposes.[370] This method, that is, with 2-mercaptoethanol, should not be used for the measurement of IgM antibodies.

When two different compounds (i.e., methanol and formalin) are used to fix parasites for use in the agglutination test, a "differential" agglutination test (AC/HS test) results because the different antigenic preparations vary in their ability to recognize sera obtained during the acute and chronic stages of the infection.[371] This test has proved useful in helping differentiate acute from chronic infections and is best used in combination with a battery of other tests. When the AC/HS yields a "nonacute" pattern, the infection has been present for at least 12 months from the time of serum sampling.[372]

Immunoglobulin G Enzyme-Linked Immunosorbent Assay

The IgG-ELISA method is now the most widely used for the demonstration of IgG antibodies to *T. gondii*. Most commercial IgG antibody test kits are accurate for demonstration of IgG antibodies; however, it is important to recognize that for patient care one cannot use a single IgG titer, no matter what its level, to predict whether the infection was recently acquired or acquired in the distant past.

Immunoglobulin G Avidity Test

A number of tests for avidity of *Toxoplasma* IgG antibodies have been introduced to help differentiate between recently acquired and distant infection.[373] This method is based on the observation that during acute *T. gondii* infection, IgG antibodies bind antigen weakly (i.e., have low avidity), whereas chronically infected patients have more strongly binding (high avidity) antibodies.[373] Protein-denaturing reagents, including urea, are used to dissociate the antibody-antigen complex. Low or equivocal avidity test results can persist for months to years after the primary infection,[373] and for this reason a low or equivocal avidity test result must not be used to determine whether the infection was acquired recently. The time of conversion from low or equivocal to high avidity is highly variable among different individuals, including pregnant women.[360,374,375] However, it has been demonstrated that once the avidity test result is high, the patient was infected at least 3 to 5 months earlier.[375] This timing depends on the method used. High-avidity test results by the IgG-VIDAS avidity test (or VIDAS [Vitek Immuno-Diagnostic Assay System] *Toxoplasma* IgG Avidity, bioMérieux), for example, have been essentially found only in pregnant women who have been infected for at least 4 months.[362,376,377]

The avidity test should only be used as an additional confirmatory diagnostic method in patients with positive and/or equivocal IgM test results or when the results of a battery of tests are equivocal or interpreted as consistent with the possibility of a recently acquired infection. Health care providers involved in the care of pregnant women should be aware that avidity testing is only a confirmatory test. It should not be used alone as a definitive test for decision making.

Immunoglobulin M Antibodies

IgM antibodies may appear earlier and decline more rapidly than IgG antibodies. IgM antibody tests have been widely used for the diagnosis of acute infection and to determine whether a pregnant woman has been infected during gestation or before conception. There has been a heightened awareness of the fact that titers in tests for IgM antibodies may persist for years after the acute infection and that the reliability of commercially available assays varies considerably.[378–380] Both the laboratory performing the test and the physician requesting the test should be aware of this problem. In 1997 the US Food and Drug Administration (FDA) issued a health advisory warning about the use of *T. gondii* IgM commercial test kits as the sole determinant of recent infection in pregnant women.[381] At present, the decision to treat or undertake other medical interventions, including the termination of pregnancy, should be based on clinical evaluation and additional testing performed in reference or research laboratories with experience in the diagnosis of toxoplasmosis. In support of this recommendation, a recent retrospective study of samples from 451 patients with positive IgM at nonreference laboratories that were analyzed at a reference laboratory between 2003 and 2013 found that only 22% had an acute infection; of the remainder, 36% were IgM positive in the context of chronic disease.[359] For further discussion, see "*Toxoplasma gondii* Infection in Pregnancy."[382] The persistence of IgM antibodies for several years appears not to have clinical significance.[9]

Immunoglobulin M Indirect Fluorescent Antibody Test

IgM-IFA antibody appears within the first week of infection; titers rise rapidly and then fall to low titers and usually disappear within a few months. Low titers may persist 1 year or longer.[383] Antinuclear antibodies and rheumatoid factor may cause false-positive results.[384] IgG-blocking antibodies can cause false-negative results in this test when IgG is not removed.[385]

Immunoglobulin M Enzyme-Linked Immunosorbent Assay

The double-sandwich IgM-ELISA for detection of IgM-specific antibodies to *T. gondii*[386–388] is currently the most widely used method for demonstration of IgM antibodies to *T. gondii* in adults, the fetus, and newborns.[9] In contrast to the conventional method, in which the wells of microtiter plates are coated with antigen, the wells are coated with specific antibody to IgM. The double-sandwich IgM-ELISA is more sensitive than the IgM-IFA test for diagnosis of recently acquired infection, and serum samples that are negative in the DT, but that contain either antinuclear antibodies or rheumatoid factor and thus cause false-positive results in the IgM-IFA test, are negative in the double-sandwich IgM-ELISA. This latter observation is attributed to the fact that serum IgM fractions are separated from IgG fractions during the initial step in the double-sandwich IgM-ELISA procedure.[9]

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. False-positive results and the problems associated with the persistence of positive titers, even years after the initial infection, remain major obstacles to correct interpretation of the results obtained in these tests.[359,379] Moreover, heat inactivation of serum (56°C, 30 minutes) can lead to decreases in IgM titers and false-negative results.[389]

Immunoglobulin M Immunosorbent Agglutination Assay

The IgM-ISAGA, which binds the patient's IgM to a solid surface and uses intact, killed tachyzoites to detect IgM antibodies, is highly sensitive.[390] The test is simple to perform, does not require the use of enzyme conjugate, and is read in the same manner as the agglutination test. Overall, it is more sensitive and specific than the IgM-IFA test. The presence of rheumatoid factor or antinuclear antibodies does not cause false-positive results in the IgM-ISAGA. In adults it is more sensitive but much less specific than the double-sandwich IgM-ELISA method. In infants the IgM-ISAGA is the most sensitive method and is used effectively for diagnosis of congenital infection in infants 6 months of age or younger.[391] A positive IgM-ISAGA test result in the first 10 days of life should be repeated after 10 days to rule out the possibility of maternal contamination of IgM antibodies.[392] The ISAGA method has also been used to detect IgA and IgE antibodies.

Immunoglobulin A Antibodies

IgA antibodies may be detected in sera of acutely infected adults and congenitally infected infants using ELISA or ISAGA.[393–395] As is true for IgM antibodies to the parasite, IgA antibodies may persist for many months or more than 1 year. However, IgA antibodies tend to disappear earlier than the IgM, and their presence at high titers is usually associated with early infections (e.g., within 3 months of the date of serum sampling). The increased sensitivity of IgA assays over IgM assays for the diagnosis

of congenital toxoplasmosis represents a major advance in the serologic diagnosis of the infection in the fetus and newborn.[395] A recent retrospective study confirmed the specificity of the ISAGA in diagnosing congenital infection.[396] IgA antibodies are rarely detectable by ELISA in sera of AIDS patients with TE.[395] If IgA antibodies are detected in the newborn during the first 10 days of life, the test should be repeated 10 days after birth to make certain that what is being measured is not contaminating maternal IgA antibodies. The possibility that such contamination might occur is the reason that, under most circumstances, peripheral blood rather than cord serum should be used to measure IgM, IgA, or IgE antibodies in the newborn.

Immunoglobulin E Antibodies

IgE antibodies are detectable by ELISA in sera of acutely infected adults,[397,398] congenitally infected infants,[397,398] and children with congenital toxoplasmic chorioretinitis.[399] The duration of IgE seropositivity is briefer than that with IgM or IgA antibodies and hence appears useful for identifying recently acquired infections.[245,398] *T. gondii*–specific IgE antibody has been detected in patients with TE and may be useful as a marker for TE in this population of patients.[398] IgE antibodies have been reported to be present in 85.7% of asymptomatic seroconverters and in 100% of seroconverters with overt toxoplasmosis.[400] For neonatal diagnosis of congenital toxoplasmosis, IgE was less sensitive than IgM and IgA, but simultaneous measurement of the three immunoglobulins at birth improved the diagnostic yield to 81%.[400] Emergence of specific IgE during postnatal treatment for congenital toxoplasmosis may indicate poor adherence or inadequate dosing.

Polymerase Chain Reaction

PCR amplification for the detection of *T. gondii* DNA in body fluids and tissues has successfully diagnosed congenital,[333,401] ocular,[212,213] pulmonary,[210] cerebral, and disseminated toxoplasmosis.[402,403] PCR of amniotic fluid has revolutionized the diagnosis of intrauterine *T. gondii* infection by enabling an early diagnosis to be made, thereby avoiding the use of invasive procedures on the fetus.[9,401] PCR has also been successfully used on samples of CSF, blood, urine, and placental and fetal tissues for diagnosis of congenital infection.[9,404–407] The sensitivity of PCR in CSF varies between 11% and 77%, whereas the specificity is close to 100%.[402] PCR may also detect the parasite in buffy coat specimens of AIDS patients with TE.[403] The sensitivity of PCR on whole blood or buffy coat ranges from 15% to 85%. PCR on blood appears to be a valuable tool primarily in patients with disseminated disease; it is less sensitive in the detection of TE because a relatively low percentage of AIDS patients with TE have parasitemia.[408,409] Prophylaxis or therapy for toxoplasmosis adversely impacts the sensitivity of the method; sensitivity is higher in CSF or blood samples collected before or within the first week of therapy.[403] In a recent case report, quantitative real-time PCR proved useful for the diagnosis and monitoring of *Toxoplasma* infection in a heart transplant recipient who developed toxoplasmosis after TMP-SMX for PCP prophylaxis had been stopped. Decreasing parasitic burdens in sequential samples of CSF, blood, and BAL fluid correlated with a favorable outcome and allowed modulation of the immunosuppressive drug regimen in this patient.[410] Peripheral blood PCR has also been found to be positive in immunocompetent patients in Brazil who present with symptomatic ocular toxoplasmosis as a result of reactivation of an infection acquired in the distant past.[411,412] It is unclear whether these findings apply only to patients in this area of the world, where atypical and more virulent strains are common in contrast to Europe and the United States.

Because there is no standardized PCR assay, performance characteristics will vary widely depending on the laboratory, gene target, primers, and sample preparation.[413] Primers targeting multicopy *B1* or *AF146527* (also known as the 529–base pair [bp] repeat element) genes appear to be the most sensitive and are the most broadly used.[413–415] A number of investigators have reported a higher sensitivity of the 529-bp target for the prenatal diagnosis of congenital toxoplasmosis when compared with the *B1* gene.[416,417] For maximal reliability, clinical samples should be sent to reference laboratories experienced in performing this assay.[406,418]

A retrospective analysis of French laboratories performing routine surveillance on blood from HSCT patients using PCR for the *AF146527* gene found that 23 of 1220 blood samples (1.9%) were positive, a lower figure than other series.[419] Fifteen patients were not receiving TMP-SMX prophylaxis and were treated for toxoplasmosis. The other eight were simply continued on TMP-SMX prophylaxis. No clinical signs of toxoplasmosis resulted. This and other studies suggest that a PCR-based preemptive strategy is useful in the earlier diagnosis and treatment of *Toxoplasma* infection, before patients develop organ involvement.[278,281,420]

Histologic Diagnosis

Demonstration of tachyzoites in tissue sections or body fluids (e.g., CSF, amniotic fluid, or BAL) establishes the diagnosis of acute infection or reactivation of a latent infection.[9] It is often difficult to demonstrate tachyzoites in stained tissue sections. Multiple tissue cysts near an inflammatory necrotic lesion can be considered as diagnostic of acute infection or reactivation of a latent infection.[421] Fluorescent antibody staining may be useful, but this method often yields nonspecific results.[422] The immunoperoxidase technique, which uses antisera to *T. gondii*, has proved both sensitive and specific; it has been used successfully in clinical settings to demonstrate the organisms in the CNS of patients with TE.[42] Both the fluorescent antibody and immunoperoxidase methods are applicable to unfixed or formalin-fixed paraffin-embedded tissue sections.[42] Fluorescein-labeled monoclonal antibodies to *T. gondii* for staining touch preparations of specimens[423] and rapid electron microscopy[424] have been used successfully to diagnose TE.

A rapid, technically simple but underused method is the detection of *T. gondii* in air-dried, Wright-Giemsa–stained slides of centrifuged (e.g., cytocentrifuged) sediment of CSF or of brain aspirate or in impression smears of biopsy tissue.

Endomyocardial biopsy has been used successfully to diagnose toxoplasmosis in heart transplant recipients.[425] Characteristic histologic criteria alone are probably sufficient to establish the diagnosis of TL in older children and adults (see "Lymph Node" under "Pathology").[197]

Isolation of *Toxoplasma gondii*

Although largely replaced by use of PCR, isolation of *T. gondii* from blood or body fluids can also be used to establish that the infection is acute. In neonates isolation of the organism from the placenta is highly suggestive of fetal involvement, and isolation from fetal tissues is diagnostic of congenital infection.[9] Attempts at isolation of the parasite can be performed by mouse inoculation or inoculation of tissue cell cultures.[9] In tissue cell cultures parasite-laden cells can be demonstrated with appropriate staining, and plaques are formed in which tachyzoites are easily recognized. Tissue cell culture has the advantage of widespread availability (e.g., virology laboratories) and yields results more rapidly (within 3–6 days) than does mouse inoculation.

Radiologic Methods

Radiologic studies are of particular help in patients with toxoplasmosis of the CNS. The presence of calcifications in the brain of a newborn, detected by radiography, ultrasonography, or CT, should heighten the suspicion of *T. gondii* as the cause of the disease (Fig. 278.6A). In severely affected infants with congenital toxoplasmosis, unilateral or, more often, bilateral and symmetrical dilatation of the ventricles is a common finding.[9]

In the majority of immunodeficient patients with TE, CT scans show multiple bilateral cerebral lesions.[426] Although multiple lesions are more common in toxoplasmosis, they also may be solitary; a single lesion should not exclude TE as a diagnostic possibility. Clinicians should be aware that toxoplasmosis may manifest as an encephalitis that at autopsy is "diffuse," in which case the neuroimaging study results may appear normal or reveal findings suggestive of HIV encephalopathy.[208]

CT scans in AIDS patients with TE reveal multiple ring-enhancing lesions in 70% to 80% of the cases.[284] In AIDS patients who are not receiving appropriate ART or anti-*Toxoplasma* prophylaxis but have detectable *Toxoplasma* IgG and multiple ring-enhancing lesions seen on CT or MRI, the predictive value for TE is approximately 80%.[427] Lesions tend to occur at the corticomedullary junction, frequently involving the basal ganglia, and are characteristically hypodense.[428,429] The number of lesions is frequently underestimated by CT, although delayed imaging after a double dose of intravenous (IV) contrast

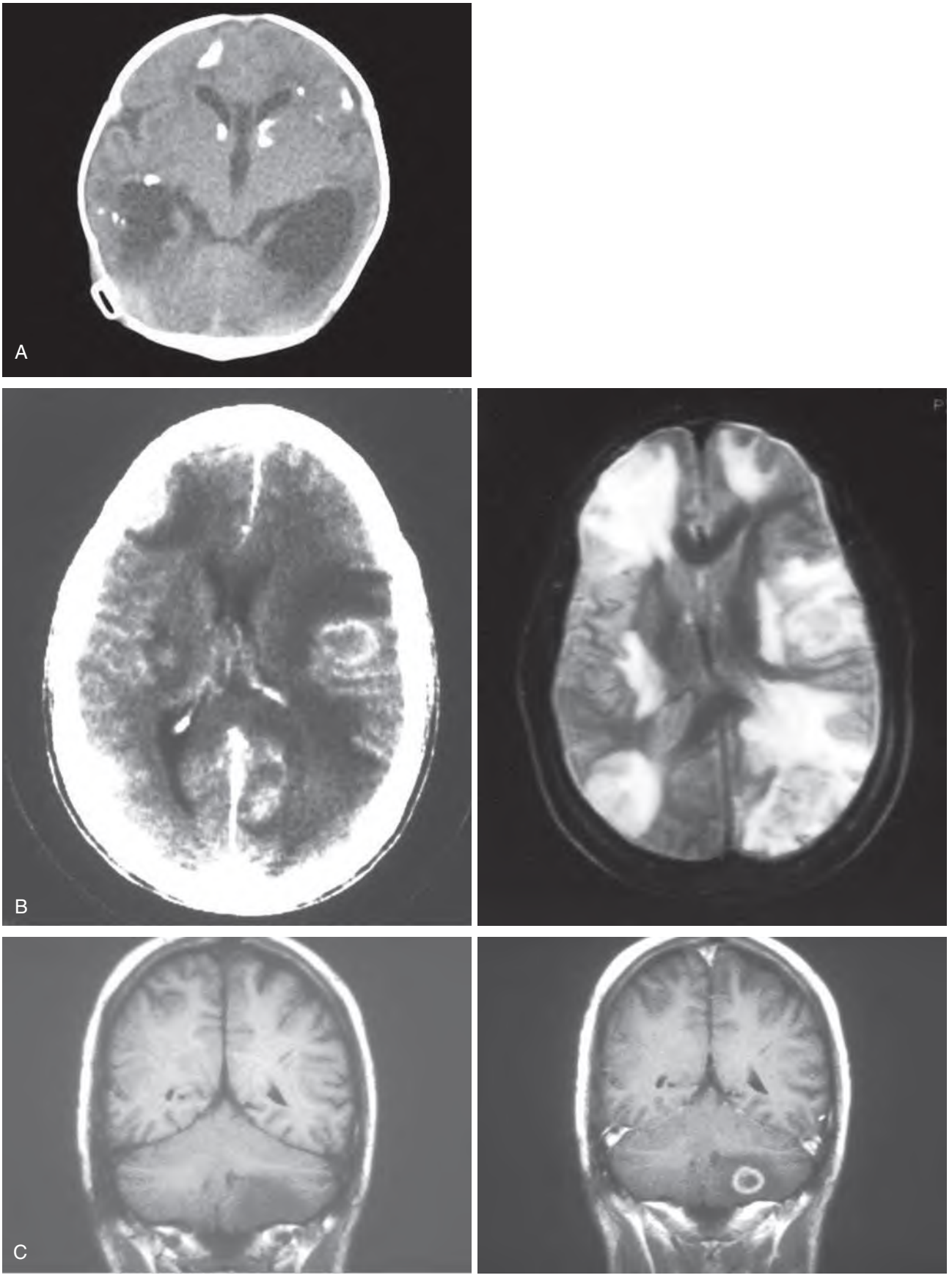

FIG. 278.6 Imaging studies of central nervous system toxoplasmosis. (A) Computed tomography (CT) scan of an infant born with congenital toxoplasmosis, illustrating the calcifications and hydrocephalus that are typically seen in the brain. (B) CT scan with contrast enhancement *(left)* and T2-weighted magnetic resonance imaging (MRI) scan *(right)* of an acquired immunodeficiency syndrome (AIDS) patient with toxoplasmic encephalitis, demonstrating multiple lesions, which are more easily identified in the MRI scan. (C) T1-weighted MRI scan without *(left)* and with *(right)* contrast enhancement of an AIDS patient with toxoplasmic encephalitis. Note the ring-enhancing lesion on the right.

material may improve the sensitivity of this modality.[428–430] An enlarging hypodense lesion that does not enhance is a poor prognostic sign.[431] TE lesions on MRI studies appear as high-signal abnormalities on T2-weighted studies and reveal a rim of enhancement surrounding the edema on T1-weighted contrast-enhanced images (see Fig. 278.6B and C). MRI has superior sensitivity compared with CT, particularly if gadolinium is used for contrast, and often demonstrates a lesion or lesions or more extensive disease not seen by CT.[430,432] Hence MRI should be used as the initial procedure when feasible or if a single lesion is demonstrated by CT. The eccentric target sign in a T1 postcontrast image is particularly suggestive of toxoplasmosis. Nevertheless, even characteristic lesions on CT or MRI studies are not pathognomonic of TE. The major differential diagnosis of focal CNS lesions in AIDS patients is CNS lymphoma, which may manifest with multiple enhancing lesions in 40% of cases. The probability of TE falls and the probability of lymphoma rises in the presence of single lesions on MRI.[426] A brain biopsy may therefore be required in the patient with a solitary lesion, especially if confirmed by MRI, to obtain a definitive diagnosis.[433] In non-AIDS immunocompromised patients, TE can also present as single or multiple ring-enhancing lesions. However, additional etiologies, including invasive fungal, nocardial, and mycobacterial infections, are more common in these patients with this radiologic presentation than in AIDS patients. Early brain biopsy and empirical treatment with a wider antimicrobial spectrum is usually required in non-AIDS immunocompromised patients with multiple space-occupying brain lesions.

In AIDS patients with TE, CT-scan improvement is seen in up to 90% of patients after 2 to 3 weeks of treatment.[426,428] Complete resolution can take from 6 weeks to 12 months; peripheral lesions resolve more rapidly than deeper ones. Smaller lesions usually resolve completely on MRI studies within 3 to 5 weeks, but lesions with a mass effect tend to resolve more slowly and leave a small residual lesion.[434] In patients receiving combination ART, lesions may persist with contrast enhancement for months to years despite adequate treatment for TE. A radiologic response to therapy lags behind the clinical response, with better correlation between them observed by the end of acute therapy.[435]

CT and MRI in toxoplasmic myelopathy usually demonstrates localized enlargement of the spinal cord,[287,436] which may result in obstruction to dye flow on myelography.[287] Gadolinium enhancement of MRI studies usually highlights as an intramedullary lesion at the site of spinal cord enlargement.[436,437]

A variety of positron-emission tomography (PET) scanning,[438] radionuclide scanning,[439] and MRI techniques[440] have been used to evaluate AIDS patients with focal CNS lesions, specifically to differentiate between toxoplasmosis and primary CNS lymphoma.[440] ^{18}F-fluorodeoxyglucose (FDG)-PET scanning is now widely used in the evaluation of patients with tumors. There is a significantly higher uptake of ^{18}F-FDG in patients with cerebral lymphoma than in patients with TE.[441] Radionuclide scanning has also been used to differentiate between CNS toxoplasmosis and lymphoma. Neoplasms usually demonstrate increased uptake of thallium 201 on both early and late scanning.[440] Although published studies suggest a high sensitivity and specificity of these studies, in practice they often are not helpful, in part because of variability in uptake and in part because they are often used after an empirical trial of anti-*Toxoplasma* therapy. Thallium scans may have decreased diagnostic utility in the setting of ART.[442]

Proton magnetic resonance (MR) spectroscopy to evaluate brain lesions has been used in a few patients. MR spectroscopy in patients with TE reveals an elevation in the lactate and lipid contents[440] and a decrease in the levels of choline. In contrast, MR spectroscopy in patients with CNS lymphoma reveals mildly elevated levels of choline.[440]

Cerebrospinal Fluid Abnormalities

CSF abnormalities in patients with TE are nonspecific; mild mononuclear pleocytosis and mild-to-moderate elevations in CSF protein are often observed, and hypoglycorrhachia is uncommon.[232,443] Almost unique to infants with neonatal toxoplasmosis, however, is the very high protein content of the ventricular fluid and eosinophilia. Although in some infants the protein level is just slightly above normal, in others it can be measured in grams per deciliter rather than in milligrams per deciliter.[9] Demonstration of intrathecal production of *T. gondii*–specific IgG or IgM in the absence of CSF contamination with blood is diagnostic of TE.[444,445]

Diagnosis of Specific Clinical Entities

The initial step in pursuing the diagnosis of *T. gondii* infection or toxoplasmosis is to determine whether the patient has been exposed to the parasite. In virtually all cases, tests for IgG antibodies reliably establish the presence or absence of the infection; a negative IgG test essentially rules out prior or recent exposure to the parasite. However, clinicians should be aware that IgG antibodies may be absent in immunocompetent patients who have been tested within the first 2 weeks of the acute infection, in patients with severe immunodeficiencies involving defective production of IgG antibodies, and in BMT patients despite having had detectable IgG antibodies in the pretransplantation period. Because of the higher sensitivity of the IgG DT, on occasion, IgG antibodies are initially negative in commercially available kits but positive in the IgG DT (see "Serologic Tests for Demonstration of Antibody"). In addition, cases of documented toxoplasmic chorioretinitis and TE in adult patients have been observed in which IgG antibodies were not demonstrable; such cases are very uncommon.

In the presence of clinical illness it is important to establish whether the patient's symptoms are due to a recently acquired infection or to recrudescence of latent infection (chronic infection) or are unrelated to the infection. A true negative IgM test in an otherwise immunologically normal individual essentially rules out that the infection has been acquired in recent months. A positive IgM test is more difficult to interpret correctly. One must not assume that a positive IgM test result is diagnostic of recently acquired infection. The presence of *T. gondii*–specific IgM antibodies can be interpreted as a true-positive result consistent with recently acquired infection, a true-positive result consistent with a chronic infection (IgM antibodies have been shown to persist in some individuals for as long as several years after the acute infection), or a false-positive result. To establish which of these is most likely in a given case, confirmatory testing in a reference laboratory should be performed whenever feasible.[359,379,381]

The use of a combination or battery of tests has been useful for determining whether the patient has been recently infected or infected in the more distant past.[245,361] If the patient has received a blood transfusion or intravenous immunoglobulin (IVIG), serologic tests may measure exogenously administered rather than endogenous antibody. The use of serologic tests to evaluate the response to therapy should be discouraged. The PAMF-TSL currently serves as a reference laboratory. Physicians in the PAMF-TSL also offer interpretation of test results and consultation on treatment and patient management to clinicians in the United States and worldwide.

Toxoplasmosis in the Immunocompetent Patient

Tests for IgG and IgM antibodies should be used for initial evaluation of immunocompetent patients. Testing of serial specimens obtained 3 weeks apart (in parallel) provides the best discriminatory power if the results in the initial specimen are equivocal. Negative results in both of these tests virtually rule out the diagnosis of toxoplasmosis. Early in infection, IgG antibodies may not be detectable, whereas IgM antibodies are present, hence the need for both tests to be performed. Acute infection is supported by documented seroconversion of IgG or IgM antibodies or a greater-than–two-tube rise in antibody titer in sera run in parallel. A single high titer of any Ig antibodies is insufficient to make the diagnosis; IgG antibodies may persist at high titers for many years,[9] and IgM antibodies may be detectable for more than 12 months. When only a single serum sample is available, a battery or combination of tests is usually required in determining the likelihood that the infection is acute.

Toxoplasmosis should be considered in the differential diagnosis of lymphadenopathy, whether or not symptoms are present and especially in those without symptoms. Confirmatory serologic tests should be obtained in such patients. The interval between the clinical onset of lymphadenopathy and the date that the specimen is drawn is critical for interpretation of the test results.[245] In patients whose serum is available during the first 3 months after the clinical onset, at least the IgG test

and the IgM-ELISA are positive, IgG avidity will be low, AC/HS will exhibit an acute pattern, and IgA and IgE may be positive. In those patients in whom sera are obtained more than 6 months after the clinical onset, the IgG will be positive, the IgM-ELISA is most likely to be negative, and the IgA-ELISA and IgE-ELISA are likely to be negative and the avidity and AC/HS maybe high and exhibit a chronic pattern, respectively.[245] A high IgG avidity test result in an individual who has recent onset of lymphadenopathy (e.g., within 2–3 months of sera sampling) suggests a cause other than toxoplasmosis,[372] and further workup is warranted. A nonacute AC/HS pattern earlier than 12 months after clinical onset of lymphadenopathy should suggest an etiology other than TL. In such cases investigation for alternative causes, including malignancy, should be undertaken.[372]

Histologic diagnosis can be useful in some cases of suspected toxoplasmosis in the immunocompetent patient. The histologic criteria for the diagnosis of TL have been well established (see Fig. 278.3A; see "Histologic Diagnosis").[197] In this setting there is no need to visualize the parasite. Endomyocardial biopsy and biopsy of skeletal muscle have been successfully used to establish *T. gondii* as the etiologic agent of myocarditis and polymyositis in rare cases in immunocompetent patients.[198] Isolation studies and PCR have rarely proved useful in immunocompetent patients.

Toxoplasmosis in the Immunodeficient Patient

Because reactivation of chronic infection is the most common cause of toxoplasmosis in patients with AIDS, malignancies, or organ transplants, initial assessment of these patients should routinely include an assay for *T. gondii* IgG antibodies. IgG antibody testing should be ideally performed as soon as it is established that the patient is immunocompromised or is about to be immunosuppressed. Those with a positive result are at risk of reactivation of the infection; those with a negative result should be instructed on how they can prevent becoming infected (see "Prevention"). Those at highest risk (e.g., HIV-infected patients not receiving appropriate ART or anti-*Toxoplasma* prophylaxis) who are initially seronegative should be retested on an annual basis to determine whether they have seroconverted. Seronegative organ transplant recipients should be identified before transplantation because they will be at risk for infection if a seropositive donor who can potentially transmit the parasite via the allograft is selected. In this setting administration of anti-*Toxoplasma* prophylaxis in the posttransplantation period can avoid unnecessary morbidity and mortality.[95]

In patients with AIDS and toxoplasmosis, the IgG titer may be relatively low, and tests for IgM, IgA, and IgE antibodies are usually negative.[284]

In the early postoperative period in heart transplant recipients with pretransplant *Toxoplasma* antibodies who present with a clinical illness, serologic test results may be misleading.[261,425] In these patients results indicating apparent reactivation (rising IgG and IgM titer) may be present in the absence of clinically apparent infection. In addition, serologic test results consistent with chronic infection may be seen in the presence of toxoplasmosis.[261,425] In heart transplant recipients in whom toxoplasmosis is suspected as a cause of altered myocardial function, endomyocardial biopsy has proved useful.[425] The parasite has been demonstrated in the myocardium of patients in whom the biopsy was performed because of a suspicion of rejection.[425] PCR testing of peripheral blood, BAL, CSF, vitreous fluid, and other body fluids or tissues may prove to be useful in patients with solid-organ transplants who are suspected to have toxoplasmosis.

Diagnosis in the BMT recipient often requires special consideration. It is critical in all BMT patients that a serum IgG titer be performed before the transplantation. Toxoplasmosis in these patients is almost always due to recrudescence of a latent infection. After BMT, the preexisting IgG antibody titer may rise, remain stable, decrease, or become negative. Thus posttransplantation serology frequently is not helpful in this group of patients and emphasizes the need for knowing the patients' pretransplantation serologic status. Clinical evidence of encephalitis, pneumonia, fever, brain lesions, or any other unexplained syndrome in BMT patients with preexisting *T. gondii* IgG antibodies must include toxoplasmosis in the differential diagnosis. The ultimate diagnosis of toxoplasmosis in these patients requires the use of histologic, cytologic, PCR, or isolation methods to detect direct or indirect evidence of the actively replicating parasite. In patients with allogeneic HSCT who are IgG antibody test positive for *T. gondii* before transplantation, routine PCR testing of peripheral blood specimens in the posttransplantation period has been proposed as an appropriate tool for guiding preemptive therapy.[281] In one report of 106 patients toxoplasmosis developed in 38% of those who were PCR positive versus 0% in those who were PCR negative.[278] In later studies, however, a first positive PCR was seen only at the time of presentation with clinical symptoms or subsequent to that.[420,446]

Serologic tests in patients with hypoglobulinemia or agammaglobulinemia may not be useful to diagnose toxoplasmosis; active infection can occur in these patients in the setting of negative IgG titers. Serologic testing in patients receiving IVIG is also potentially misleading because positive titers may reflect those of the individuals in the donor pool.

A definitive diagnosis of toxoplasmosis in the immunodeficient patient relies on histologic demonstration of the parasite (usually in association with an inflammatory process), on detection of *T. gondii* DNA by PCR, or on isolation of the parasite. PCR or attempts to isolate the parasite can be performed in essentially any body fluid or tissue that is clinically affected. Peripheral blood testing by PCR (and by isolation) should be considered in immunocompromised patients as diagnostic of disseminated toxoplasmosis. The visualization of tachyzoites is diagnostic of active infection regardless of tissue or body fluid. The presence of a solitary *T. gondii* tissue cyst may only reflect chronic infection unless it is associated with an area of inflammation (e.g., as seen in myocardial biopsy); however, visualization of several tissue cysts virtually always means that active infection is present.

When clinical signs suggest involvement of the CNS or spinal cord, the workup should include CT or MRI (see "Radiologic Methods") of the brain. These studies should be performed even if the neurologic examination does not reveal focal deficits.

A lumbar puncture should be performed if it can be done safely, ideally before or soon after initiation of therapy; PCR can then be performed on the CSF specimen. A positive *Toxoplasma* PCR essentially confirms the diagnosis of TE. CSF can also be sent for isolation studies if available. PCR examination of the CSF also can be used for the detection of Epstein-Barr virus, JC virus, or CMV DNA in patients in whom primary CNS lymphoma, progressive multifocal leukoencephalopathy, or CMV ventriculitis, respectively, have been entertained in the differential diagnosis. Especially in HIV-infected patients, a positive Epstein-Barr virus PCR in the setting of a focal contrast-enhancing CNS lesion is strongly suggestive of CNS lymphoma.[447] Demonstration of the intrathecal production of *T. gondii*–specific antibody within the CSF may help confirm the diagnosis of TE.[445] Unless sufficient CSF is available, we suggest that the highest priority be for PCR and an attempt at isolation of the parasite. Detection of *T. gondii*–specific IgM has a very low yield and is not recommended.

Empirical anti–*T. gondii* therapy for patients with multiple ring-enhancing brain lesions (usually established by MRI), positive IgG antibody titers against *T. gondii,* and AIDS (e.g., patients with a CD4 count of <200 cells/mm^3) is accepted practice; a clinical and radiologic response to specific anti–*T. gondii* therapy essentially confirms the diagnosis of TE (Fig. 278.7). Use of adjunctive corticosteroids to decrease cerebral edema can cause temporary clinical improvement that is incorrectly attributed to the pyrimethamine and sulfa. This empirical approach is not recommended for non-AIDS immunocompromised patients with this presentation because the differential diagnosis is wider and often includes other etiologic agents, such as invasive molds, nocardiae, bacterial brain abscess, and mycobacteria. In these patients an early brain biopsy is recommended. Brain biopsy should be considered in AIDS patients with presumed TE if there is a single lesion on MRI, a negative IgG antibody test, an inadequate clinical response (within a 2- to 3-week period) or progression during optimal therapy, or in patients the physician considers to have adhered to an effective prophylactic regimen against *T. gondii* (e.g., TMP-SMX). An impression smear of the brain biopsy specimen can be made and immediately examined for the presence of tachyzoites with the conventional Wright-Giemsa stain for blood smears as used in most laboratories. In addition to H&E staining, *T. gondii*–specific immunoperoxidase staining should be performed. Because the amount of brain tissue obtained at aspiration

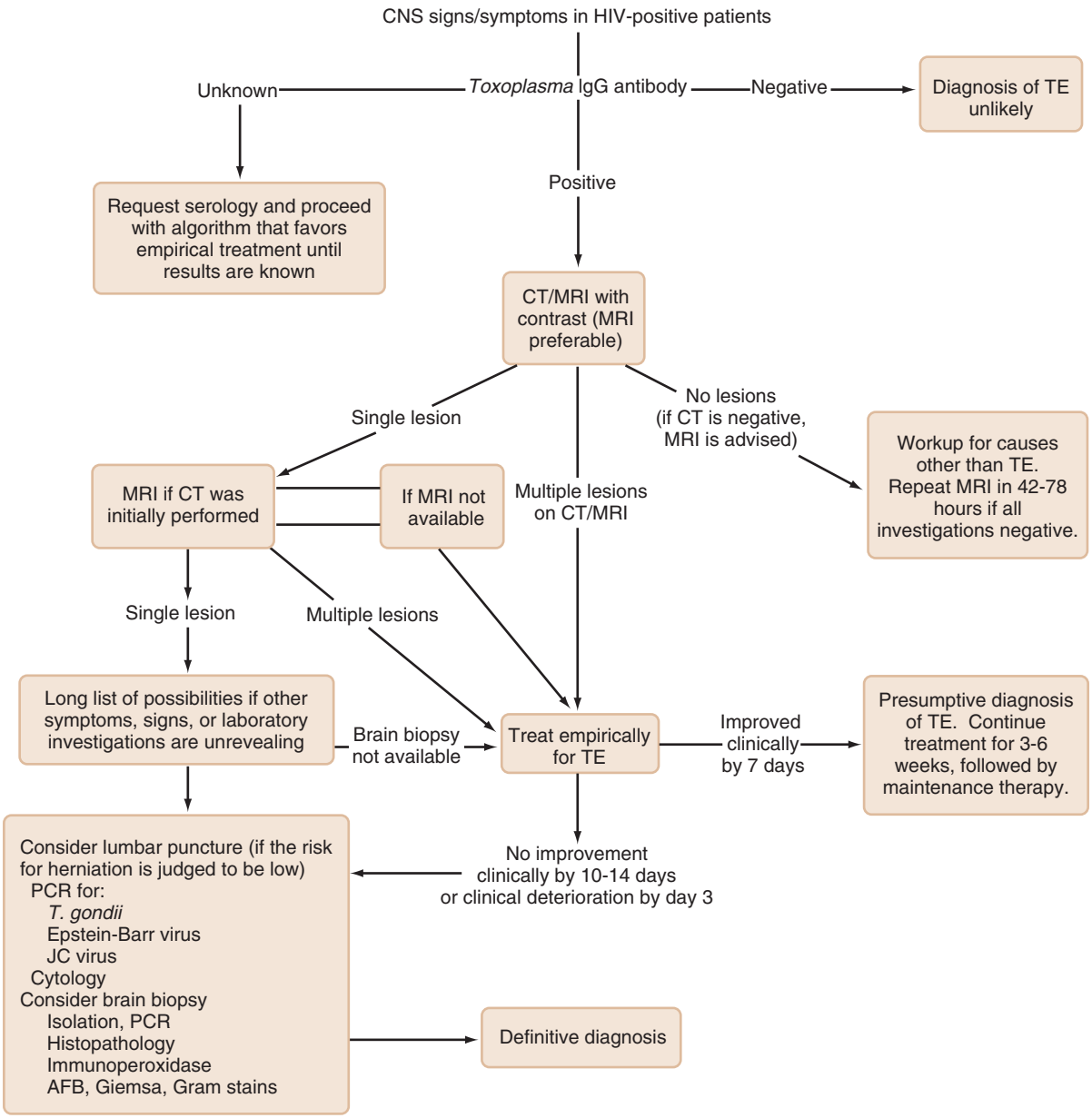

FIG. 278.7 Diagnostic approach and management algorithm for human immunodeficiency virus *(HIV)*-infected patients with central nervous system *(CNS)* symptoms or signs that might potentially be toxoplasmic encephalitis *(TE).* *AFB,* Acid-fast bacilli; *CT,* computed tomography; *IgG,* immunoglobulin G; *MRI,* magnetic resonance imaging; *PCR,* polymerase chain reaction.

or biopsy is usually small, sufficient tissue for mouse inoculation may not be available; however, this should be performed whenever feasible. A positive result may often be obtained with far less than 1 g of brain tissue. PCR has been used successfully in brain tissue to diagnose TE,[448] but a positive result should be interpreted with caution because it may not distinguish between the patient with TE from one with latent infection (asymptomatic carrier of brain tissue cysts) who has CNS pathology resulting from a process other than toxoplasmosis.

In the appropriate clinical setting it is important to include toxoplasmosis in the differential diagnosis of pulmonary symptoms, particularly in those individuals with interstitial infiltrates or ground-glass opacities. Wright-Giemsa staining and PCR of BAL specimens are useful for the diagnosis of pulmonary toxoplasmosis.[210,449]

In patients with visual symptoms in whom toxoplasmic chorioretinitis is a possibility, PCR examination of vitreous or aqueous fluid can be considered and is particularly helpful in patients with atypical clinical features of toxoplasmic chorioretinitis and in immunocompromised patients.[213,320,450]

The intraocular production of *T. gondii*–specific IgG antibodies has also been reported to be diagnostically useful.[451] Local antibody production in aqueous humor can be quantified by calculating the Goldmann-Witmer (GW) coefficient. The GW coefficient expresses the level of *Toxoplasma*-specific IgG relative to the level of total IgG in the aqueous humor as a fraction of the level of *Toxoplasma*-specific IgG relative to the level of the total IgG in the serum.[452] The GW coefficient = *Toxoplasma* IgG/total IgG in aqueous humor ÷ *Toxoplasma* IgG/total IgG in serum. A coefficient greater than 2 is considered positive and diagnostic of ocular toxoplasmosis. It has been well established that, in contrast to ocular viral infections, the GW coefficient is a more sensitive method than PCR for the diagnosis of ocular toxoplasmosis. However, PCR in vitreous fluid appears to be superior to the GW coefficient in immunocompromised patients, the elderly, and those with atypical lesions and extensive retinochoroiditis.

PCR and isolation studies in peripheral blood can help establish *T. gondii* as the etiologic agent of a febrile syndrome or systemic symptoms of unclear cause.[403,453] These studies tend to have a higher yield early in

the disease and before or shortly after specific anti–*T. gondii* therapy is initiated.[403]

Ocular Toxoplasmosis

In patients with active chorioretinitis caused by reactivation of congenital *T. gondii* infection, low titers of IgG antibody are usual, and IgM antibodies are not usually detected. In contrast, in patients with chorioretinitis as a result of an acute infection, IgG and IgM antibodies will be detected.

In most cases toxoplasmic chorioretinitis is diagnosed by ophthalmologic examination, and empirical therapy directed against the organism is often instituted based on clinical findings and serologic test results. In a number of patients the morphology of the retinal lesion or lesions may be nondiagnostic, or the response to treatment may be suboptimal, or both. In such cases (unclear clinical diagnosis or inadequate clinical response, or both) the detection of an abnormal *T. gondii*–antibody response in ocular fluids (GW coefficient),[450,454] or demonstration of the parasite by isolation, histopathologic examination, or PCR have been used successfully to establish the diagnosis.[452,455] PCR has been used in both vitreous and aqueous fluids in an attempt to support or confirm the diagnosis of *T. gondii* as the cause of the retinal lesions.[212,213,320,450]

Toxoplasma gondii Infection in Pregnancy

Acute acquired *T. gondii* infection is diagnosed serologically by the same methods used for immunocompetent adults discussed earlier (Fig. 278.8).[382] Special care is taken to determine whether the infection was acquired before or after conception. This determination is frequently difficult because routine serologic screening is not conducted in pregnant women in the United States.

The diagnosis of acute *T. gondii* infection or toxoplasmosis ideally requires demonstration of a rise in titers in serial serum samples, either conversion from a negative to a positive titer or a fourfold rise from a low to a higher titer.[9] These specimens should be obtained at least 3 weeks apart and be tested in parallel. Because the diagnosis is frequently considered relatively late in the course of the patient's pregnancy, serologic test titers may already have reached their peak at the time the first serum is obtained for testing. Therefore it is often difficult to discriminate between infections acquired recently (possibly during pregnancy) and those acquired in the more distant past, when the only available serum sample has been obtained in the third trimester of the pregnancy. Thus the initial serum should be obtained as early as possible during gestation.

Initial screening of maternal serum involves testing for IgG and IgM antibodies; a lack of both Ig antibodies essentially excludes active infection but identifies the patient as being at risk for acquisition of the infection and hence in need of instruction about primary prevention. The presence of IgG antibodies in the absence of IgM antibodies in the first two trimesters almost always indicates chronic maternal infection with essentially no risk to the fetus; the exceptions are severely immunodeficient patients. In the third trimester a negative IgM test titer is most likely consistent with a chronic maternal infection but does not exclude the possibility of an acute infection acquired early in pregnancy; this is especially true in those patients who exhibit a rapid decline in their IgM titers during the acute infection. In these cases the use of other serologic tests (e.g., IgA, IgE, AC/HS, avidity) may be of particular help.

A positive IgM test result requires further assessment with confirmatory testing at a reference laboratory (see also "Diagnosis of Specific Clinical Entities").[361,379,381,382] The use of confirmatory testing with a combination of serologic tests in a reference laboratory has proved helpful in discriminating between recently and more distantly acquired infections, and having an expert interpret the results to the patient's physician has been shown to reduce unnecessary induced abortions among pregnant women reported to have IgM antibodies.[361] Women who are informed that they have a positive IgM test titer and that it signifies that their offspring will or might be infected often choose abortion. Unfortunately, a positive IgM test may not necessarily indicate infection acquired during gestation (a false-positive result or persistence of a IgM-positive result in the chronic stage of the infection), and thus the abortion may not be indicated.[389] It is for this reason that confirmatory testing in a reference laboratory has been recommended by many experts and by the FDA.[381]

A number of tests for avidity of *Toxoplasma* IgG antibodies have been introduced to help differentiate between recently acquired and distant infection.[373,374,456] 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 have been infected with *T. gondii* for at least 3 to 5 months. Recently, it has been demonstrated that, when used as a confirmatory test, along with a battery of other tests, in women in their first 16 weeks of gestation, detection of high-avidity IgG antibodies by the IgG-VIDAS avidity test, for example, can be a useful addition to the discriminatory power of a combination of tests in distinguishing recently acquired from chronic infection.[357,362,377] It is critical to recognize that the value of the avidity test is in the first 3 to 5 months of gestation, that is, the fetus of a woman in the 14th week of gestation with a positive IgM test and a high-avidity result is not at risk for congenital toxoplasmosis.[362] Because low or equivocal avidity test results may persist for many months, their presence does not necessarily indicate recently acquired infection.

Confirmatory testing, using a battery of tests and the VIDAS avidity method in pregnant women during their first 16 weeks of gestation, has the potential to decrease the need for follow-up sera and thereby reduce costs, to make the need for PCR on amniotic fluid and for treatment of the mother with spiramycin unnecessary, to remove the pregnant woman's anxiety associated with further testing, and to decrease unnecessary abortions.[360–362] Although the avidity test represents an additional confirmatory method (most useful if high-avidity antibodies are detected within the first 16 weeks of gestation), it should not be used as the only confirmatory test for pregnant women with positive IgG and/or IgM antibodies because of the potential to misinterpret low- or borderline-avidity antibody results.

Once the diagnosis of acute acquired infection during pregnancy has been presumptively established, diagnostic efforts should focus on determining whether the fetus has been infected.

Congenital Infection in the Fetus and Newborn

Prenatal diagnosis of fetal infection is advised when a diagnosis of acute infection is established or highly suspected in a pregnant woman. Methods to obtain fetal blood, such as periumbilical fetal blood sampling, have been largely abandoned because of the rate of false-negative prenatal diagnoses, the risk involved for the fetus, and the delay in obtaining definitive results with conventional parasitologic tests.[333]

Prenatal diagnosis of congenital toxoplasmosis is presently based on ultrasonography and amniocentesis. PCR on amniotic fluid for the detection of *T. gondii*–specific DNA performed at 18 weeks of gestation or later is more sensitive, more rapid, and safer than conventional diagnostic procedures involving fetal blood sampling.[333,457] Amniotic fluid should be tested by PCR in all cases, with serologic test results diagnostic of, or highly suggestive of, acute acquired infection during pregnancy, and also if there is evidence of fetal damage by ultrasound examination (e.g., hydrocephalus and/or calcifications). In a prospective cohort study conducted in France, amniotic fluid PCR was performed in 261 women who had been identified as having an acute infection during gestation through routine prenatal screening for toxoplasmosis.[457] Overall sensitivity and negative predictive value of the amniotic fluid PCR for the diagnosis of congenital infection were 92.2% (95% CI, 81% to 98%) and 98.1% (95% CI, 95% to 99.5%), respectively. Specificity and the positive predictive value were 100%.[457] Overall sensitivity has been reported by several groups as varying from 65% to 92.2%; specificity and the positive predictive value have been reported to be 100% by most groups.[413,458] Recently, one group proposed a novel and more realistic interpretation of amniotic fluid PCR test results according to the pretest probability of infection and gestational age at the time of maternal infection[458]; their analysis revealed that negative test results are more significant than previously thought, given that transmission occurs only in a percentage of women at various stages of gestation and that a negative amniotic fluid PCR further reduces this percentage. Of note, the vast majority of their pregnant women, as is the case for all studies reported from Western Europe, received the benefit of prenatal treatment. Thus caution should be exercised when attempting to apply risks of congenital infection to infants born to women who were not treated during gestation. The preferable time for amniocentesis is at 18

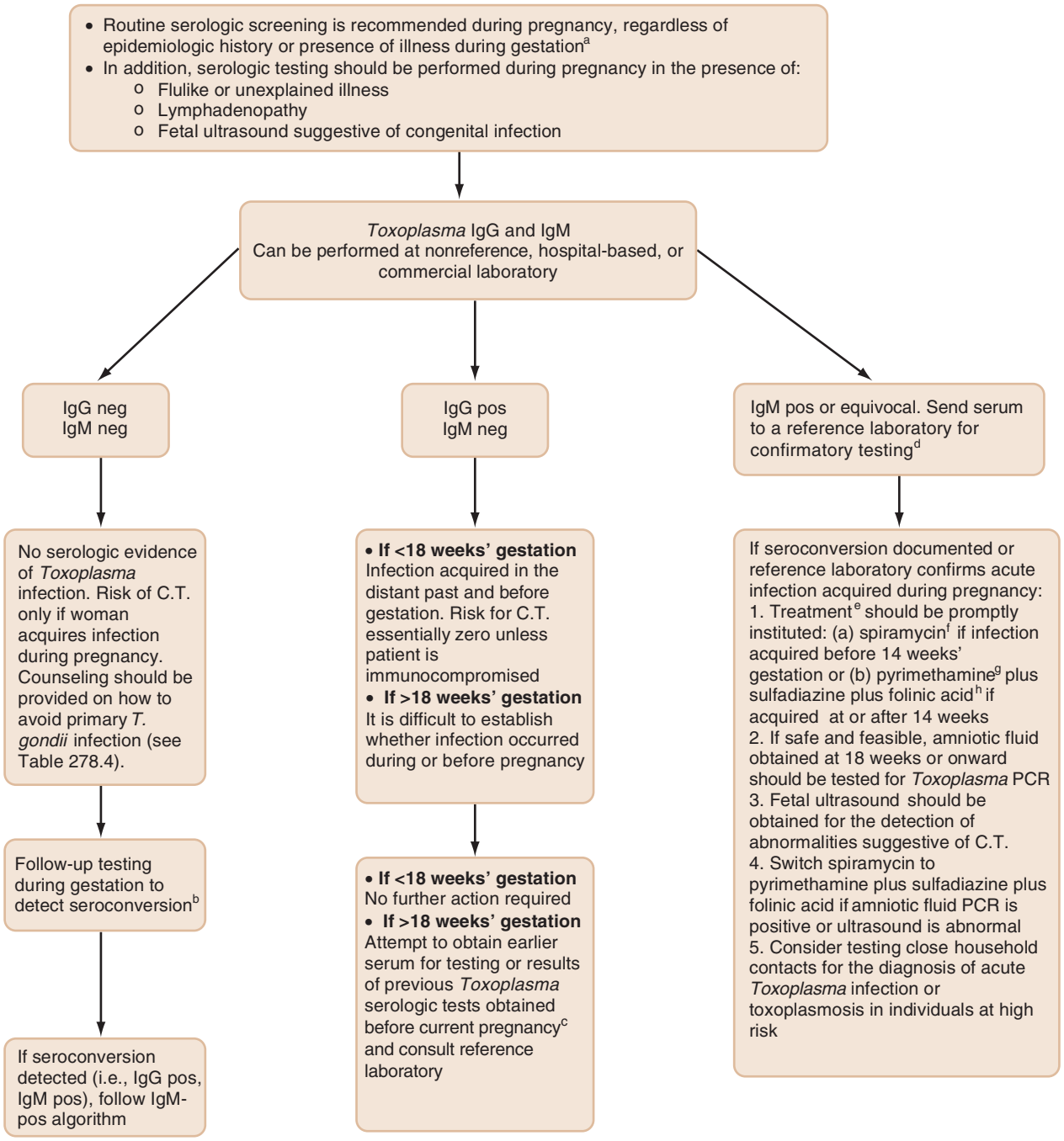

FIG. 278.8 Diagnostic approach and management algorithm of toxoplasmosis during pregnancy. Most of the initial serologic screening can be accomplished by nonreference or commercial laboratories. Only positive immunoglobulin M results should be considered for additional testing and consultation with medical experts at a reference laboratory. *C.T.,* Congenital toxoplasmosis; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *neg,* negative test result; *pos,* positive test result. [a]Up to 50% of women who acquire *Toxoplasma* infection during gestation do not have a known risk factor for acute infection or an illness suggestive of toxoplasmosis. Thus, to identify all women at risk, serologic screening should be performed in all pregnant women, along with other routine screening tests. [b]In a recent study from Lyon, France monthly screening of seronegative pregnant women was reported to significantly decrease the risk of vertical transmission and of clinical signs at 3 years of age.[558] [c]Consider consultation with a physician expert in management of toxoplasmosis during pregnancy (e.g., in the United States, Palo Alto Medical Foundation–*Toxoplasma* Serology Laboratory [PAMF-TSL], www.pamf.org/serology/; 650-853-4828; e-mail, toxolab@pamf.org or US [Chicago] National Collaborative Treatment Trial Study [NCCTS]; 773-834-4152). [d]Consider sending serum sample to a reference laboratory (e.g., PAMF-TSL). [e]Treatment regimens vary by country. The pyrimethamine-sulfadiazine–folinic acid regimen should not be offered to any pregnant women before 12 weeks of gestation because of potential teratogenicity. In some centers in Europe this regimen is offered at 14 weeks of gestation or later; in the United States it is recommended at 14 weeks or later. [f]Spiramycin is not commercially available in the United States. It can be obtained at no cost and after consultation with PAMF-TSL or the NCCTS through the US Food and Drug Administration. [g]When using pyrimethamine, folic acid should be discontinued from the prenatal multivitamins. Folic acid can potentially counteract the antiparasitic effect of the drug. [h]Folic acid should not be erroneously used instead of folinic acid.

weeks of gestation or later if acute infection was acquired after 18 weeks. The sensitivity of the amniotic fluid PCR test appears to be lower when the amniocentesis is performed before 18 weeks of gestation[333] and should be avoided before 18 weeks for the purpose of diagnosing congenital toxoplasmosis.

Maternal IgG antibodies present in the newborn may reflect either past or recent infection in the mother. For this reason, tests for the detection of IgA and IgM antibodies are commonly used for the diagnosis of infection in the newborn (Fig. 278.9).[392] It is essential that maternal contamination of blood obtained at birth be excluded; serum samples