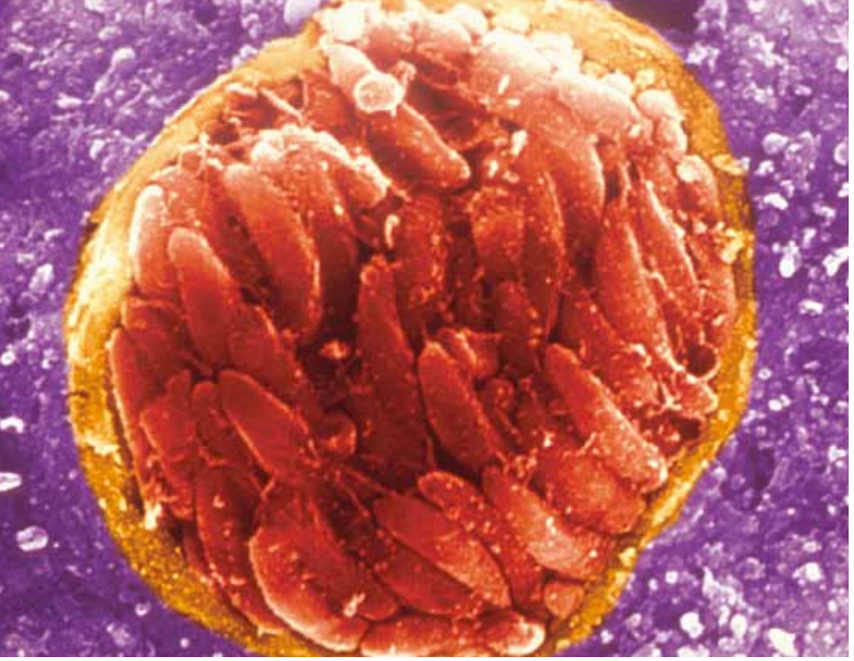
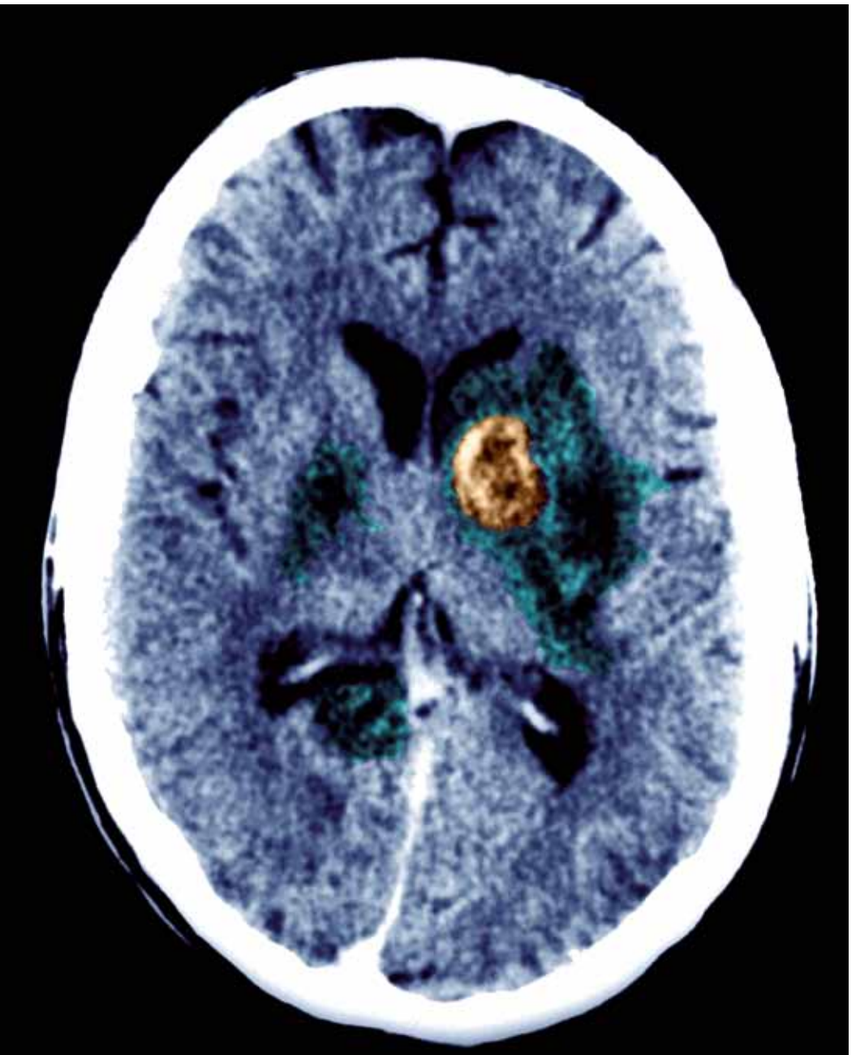




Toxoplasma gondii (Fig. 1) is a protozoan parasite that can be transmitted directly from cats to humans through faecal contamination of food, or indirectly from cats to livestock and then to humans through undercooked meat. Around 30% of humans in the United Kingdom are infected, and as such, harbour dormant cysts in their brain, but few have overt symptoms of disease. Neurological disease can occur in these people if they become immunosuppressed (Fig. 2). The possibility that apparently healthy people with infection are more likely to develop psychiatric disease, including schizophrenia and depression, is under investigation. Infection during pregnancy can cause abortion or foetal infection. Congenital disease can result in systemic, neurological and progressive eye disease. No vaccine exists for prevention of infection or disease and current drug treatments are not entirely effective.

100 years of *T. gondii* research

T. gondii (Fig. 1) was discovered about 100 years ago in a rodent in North Africa by Nicolle and Manceaux, and later the same year in Brazil as an infection in a rabbit by Splendore. The protozoan was initially thought to be a new species of *Leishmania* (originally *Leishmania gondii*, but it was soon realized to be an entirely new entity). Its name was derived from the Greek words *toxon* (bow- or crescent-shaped) and *plasma* (cell). Studies into the morphology of *T. gondii* by electron microscopy began in the 1950s, and the complete life cycle with the identification of the feline family as the definitive host was only elucidated in the 1960s. Hutchison and his team from the University of Strathclyde in Glasgow played a crucial role in the discovery of the sexual cycle occurring in the intestinal tissue of the cat. Since then, *T. gondii* has become a model parasite to dissect host–pathogen relationships and the immune system.

▲ Fig. 1. A cyst containing tachyzoites bradyzoites of *T. gondii*. *F.L. Henriquez*

◀ Fig. 2. Coloured computed tomography (CT) scan of the brain of an AIDS patient with toxoplasmosis (orange area). *Sovereign, ISM / Science Photo Library*

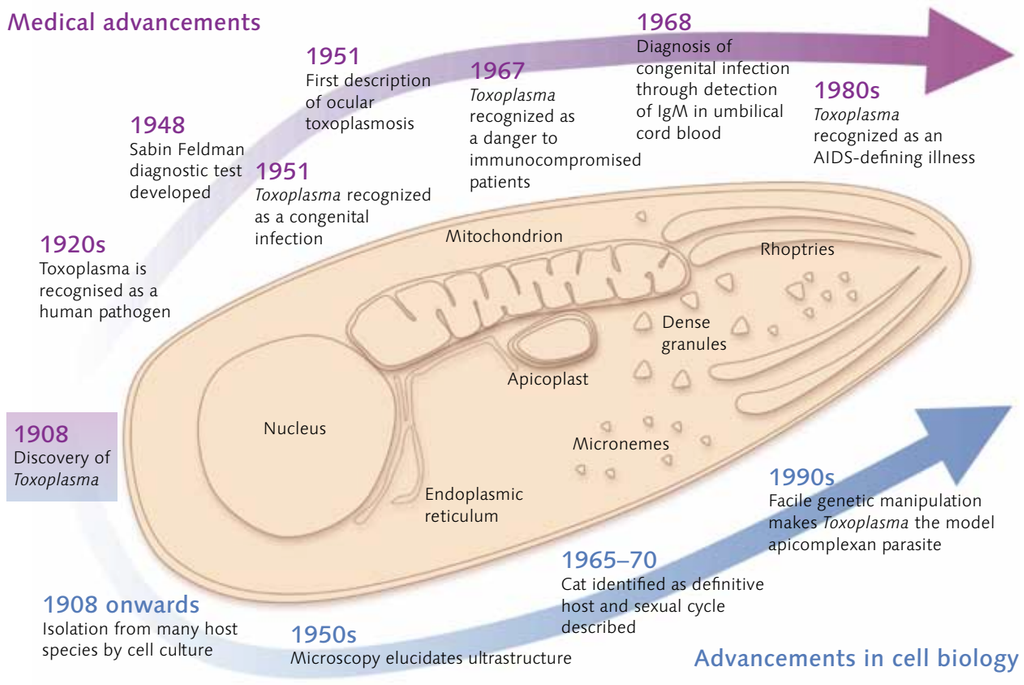
T. gondii was recognized as a human pathogen in the early 1920s. Initial observations could not identify the parasite, but in 1939 the first cases of human toxoplasmosis were described. In the same year, Albert Sabin isolated *T. gondii* from two patients, one of which was the virulent type I RH strain, named after the patient's initials and used in many laboratories worldwide to this day. Sabin and Harry Feldman developed a serological test in 1948, advancing diagnosis in humans dramatically. The severity of the disease during pregnancy was recognized in the early 1950s, with a detailed account of fatal toxoplasmosis cases in infants with hydrocephalus. Studies into congenital transmission elucidated that mother seroconversion during each trimester impacts on the severity of foetal infection, with primary infection during the first two trimesters the most damaging. Until the late 1960s, the diagnosis of congenital infection depended on seroconversion of the mother, but IgM detection in the umbilical cord greatly improved the diagnosis of congenital toxoplasmosis. Congenital disease is usually associated with post-natal brain disorders and ocular toxoplasmosis. The recognition of ocular toxoplasmosis, as a result of adult-acquired infection, was described by Reiger in 1951. The danger of *T. gondii* infection in immunocompromised patients was described in the late 1960s, but it was not until the 1980s that reactivation of toxoplasmosis was seen as critical if not treated in immunocompromised patients with chronic HIV infection (Fig. 3).

Epidemiology

The incidence of *T. gondii* infection varies considerably in humans according to geographical region. In the UK, the incidence has been reported as around 30%. In contrast, it is significantly greater in other European countries (e.g. France, 80%; Austria, 50%). In these countries, antenatal screening is compulsory and in recent years a decrease in cases has been reported. High incidence in certain European countries has been attributed to differences in food preparation with the increased risk being thought to be due to eating

A century of *Toxoplasma gondii* research

Cats are the main source of the parasite *Toxoplasma gondii* which infects many people but rarely causes disease. **Fiona L. Henriquez** and **Craig W. Roberts** describe the harmful effects of toxoplasmosis on the unlucky few and the latest scientific research into this fascinating microbe.



▲ Fig. 3. A century of *Toxoplasma* research. *F. L. Henriquez*

undercooked meat. In spite of the high level of infection in adults in some European countries, the occurrence of clinical disease is generally not perceived as a significant problem. This is at least partially due to the relatively avirulent nature of the strains of *T. gondii* causing infection in Europe. However, the general increased exposure in these countries poses a greater risk to expectant mothers and their foetuses. This is borne out by the statistics for congenital infection, which is reported as 1 in 2,000 births in Scotland, but as high as 1 in 500 births in France. Atypical and/or recombinant strains of *T. gondii* are associated with significant disease manifestations, including pulmonary involvement, splenomegaly and ocular disease in a number of non-European countries, most notably Brazil.

Disease

Disease outcome is dependent on a number of factors, including host genetics and immune status, parasite strain and mode of transmission. *T. gondii* infection in the immune-competent host is generally not seen as a major problem. Mild 'flu-like symptoms are experienced at the onset of infection which coincide with the presence of the rapidly dividing tachyzoite form of the parasite. As the immune system controls the tachyzoite stage, the parasite transforms to the bradyzoite form that encysts in various tissues throughout the body, predominantly in the brain (Fig. 2). These tissue cysts, in spite of being long-lived, are generally not perceived to cause overt disease. However, data are emerging that the cysts might contribute to psychiatric disease, including schizophrenia and depression. More subtle effects have been reported in humans, including reduced reaction times and personality changes.

Immunocompromised people, such as those infected with HIV or undergoing immunosuppressive therapy, who are infected with *T. gondii* can develop severe systemic, ocular or most commonly neurological disease. This can be due to reactivation of a chronic infection or due to a newly acquired infection.

The severity of congenital infection varies according to several factors, including time in gestation at which infection occurs, parasite strain and most probably host genetics. Foetuses infected early in gestation tend to be severely affected with overt neurological and ocular involvement at birth, whereas those infected late in gestation may not exhibit disease at birth. Essentially, all of these individuals will develop ocular lesions at some point in their life, usually during puberty. Again, the long-term management of these people is difficult due to the previously mentioned limitations in current chemotherapeutics.

Eukaryotic microbe dependent on 'prokaryotic' biochemical processes

T. gondii is a eukaryotic pathogen that has been shaped through endosymbiotic events and evolution into a 'mosaic' made up of multiple components and processes derived from eukaryotic and prokaryotic organisms. Thus in addition to the commonly found mitochondrion (derived from an alphaproteobacterial endosymbiont), *T. gondii* has an apicoplast organelle, which was obtained through secondary endosymbiosis, most probably of a red alga. Consequently, *T. gondii* has a number of biochemical processes normally found in plants and or prokaryotes, including type II fatty acid biosynthesis, isoprenoid biosynthesis and haem biosynthesis. As these are generally absent, or evolutionarily distinct from the mammalian host, they have received a great deal of interest as potential antimicrobial targets. In addition, a number of drugs with known efficacy against *T. gondii* are now known to target prokaryote-like targets within the parasite. For example, drugs such as ciprofloxin, clindamycin and spiramycin target prokaryotic DNA replication.

Model apicomplexan and a tool to understand other important related pathogens

T. gondii is evolutionarily related to a number of other important human pathogens including *Cryptosporidium* and *Plasmodium* (the parasite that causes malaria). *T. gondii* has proved to be one of the most tractable organisms to study and has consequently shed light on these related pathogens. It has sometimes been used as a surrogate experimental system. The *T. gondii* research community has also developed some systems that have been directly applied to, or altered to work in other pathogen experimental systems. Thus it is now possible to perform targeted gene deletion, episomal expression, and inducible gene knock-down, and produce parasites containing reporter constructs, such as green fluorescent protein (GFP).

Immunological lessons

The immune response to *T. gondii* is complex and multifaceted. The organism has several pathogen-associated molecular patterns (PAMPS) that interact with Toll-like receptors (TLRs) in the mammalian host to initiate an rapid immune response by innate immune cells, such as dendritic cells and macrophages. IL-12 produced by these cells stimulates natural killer (NK) cells to produce IFNγ which in turn acts on infected cells to kill parasites through the induction of reactive nitrogen intermediates, or restrict their growth through selective depletion of tryptophan, which is required by the parasite. These initial interactions control parasite replication, but are not sufficient to provide complete protection – for this, T cell activation and expansion are required. T1 helper cells, which also provide IFNγ, and cytolytic CD8 T cells, which can specifically recognize and kill infected cells, play an important role in mediating long-term immunity. Antibodies might also have a minor role in preventing invasion of the host cell.

Current treatments and future prospects

Most people receive antifolate therapy which normally comprises a combination of pyrimethamine and sulphadiazine. This is usually administered with folinic acid to reduce bone marrow toxicity. Several other therapies have been used, but none of these are able to eliminate the cystic stages. Targeting the 'prokaryotic' processes mentioned previously may offer better drugs.

Vaccine prospects

A vaccine has been sought for many years. Not surprisingly, studies have been technology-dependent (and arguably driven). Early studies used killed or homogenized parasites, or attempted attenuation. This was followed by crude extracts and then ever-increasingly enriched or purified parasite components. The advent of recombinant DNA technology allowed parasite proteins to be expressed and tested in experimental systems. Ultimately, synthetic peptides were tested in some systems. During this time, when technology allowed progressively more defined and pure antigenic components to be produced, it was noted that immunogenicity was markedly reduced. To some degree this instigated vaccine adjuvant research, but also encouraged people to contemplate viral delivery systems, naked DNA vaccination or a return to parasite attenuation. Notably, a tissue culture attenuated strain of *T. gondii* (S48) has been used as a commercial vaccine for livestock. Although such a vaccine would never be used in humans, a defined, rationally attenuated parasite produced through gene deletion techniques, resulting in auxotrophic mutants, has been very successful in murine models of infection.

Looking to the future, vaccine prospects for humans are improving. Specifically, now the *T. gondii* genome is essentially fully sequenced, and all potential antigenic peptides are 'known' and available to study. This information combined with ever more sophisticated predicative algorithms capable of predicting T cell epitopes and their interaction with various MHC alleles may allow a return to synthetic peptide vaccines with modern potent vaccine adjuvants. The challenge will be to produce a vaccine that copes with the polymorphisms in human MHC molecules at a population level and polymorphisms evident in antigenic epitopes of the different strains of *T. gondii*.

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Further reading

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