THE DEVELOPING RELATIONSHIP BETWEEN THE APICOMPLEXAN *TOXOPLASMA GONDII* AND SCHIZOPHRENIA WITH EMPHASIS ON PARASITIC HOST INVASION

By

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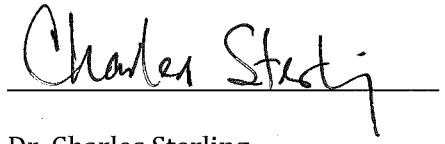

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ABSTRACT

A growing correlation is developing between parasitic infection by *Toxoplasma gondii* and the subsequent acquisition of schizophrenia. Approximately twenty-four million people worldwide suffer from schizophrenia, a disease that requires life-long treatment by means of counseling and medication to control symptoms. Although this condition affects countless individuals, the cause of schizophrenia is still unknown. But what if there was an alternate way to treat schizophrenics and conceivably even prevent the development of the disease in the first place? This possibility has been the basis of research for dedicated researchers for over half a century, all focused on one potential cause: a parasite by the name of *Toxoplasma gondii*. A projected fifty percent of the global population is seropositive for T*oxoplasma,* earning it the title of the most successful protozoan parasite on Earth. This literature review summarizes the diseases toxoplasmosis and schizophrenia before examining this promising connection. An emphasis on the mechanisms this parasite uses to invade the host provides a better basis of understanding for the reader and possibly presents a premise on how to prevent initial infection.

The Developing Relationship between the Apicomplexan *Toxoplasma gondii* and Schizophrenia with Emphasis on Parasitic Host Invasion

Hollie Mae Mills
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INTRODUCTION

Schizophrenia is a mental disorder that has afflicted the human population for hundreds of years but has only recently been recognized in the medical field as an explicit disease. This illness is characterized by the difficulty to experience normal emotional responses, think logically, recognize what is and what is not real, and function naturally in social conditions (1). The only treatments currently available for schizophrenics are a variety of medications, all which can produce life-threatening side effects that simply alleviate symptoms rather than rid the patient of the disease. This means that discontinued use of medications after years of treatment can cause patients to rapidly revert to their original condition. In other words, prognosis for those suffering from schizophrenia is unpredictable and frequently grim (1).

But what if there was alternative way to treat schizophrenia, and perhaps even prevent the development of the disease in the first place? This possibility has been the basis of research for devoted investigators for over half a century, all focused on one potential cause: a parasite by the name of *Toxoplasma gondii*.

Toxoplasma gondii is a thoroughly studied, apicomplexan protozoan that is classified by its anterior anatomy used for cell invasion. This parasite desires only a single definitive host, cats, but can also unintentionally cause infection in several other animal species, such as rodents, cattle, birds, and even humans. Typical symptoms of the parasitic disease known as toxoplasma are: frequent eye problems, severe migraines, and persistent muscle pain. This organism is also responsible for stillbirths, abortions, and deformed infants due to placental transmission from infected mothers to fetuses. But it is the more uncommon symptoms that captivate inquisitive scientists.

Toxoplasmosis has been observed to cause alterations in behavioral and neurological functions of certain animal species which highly resemble schizophrenia. In laboratory rodents, *Toxoplasma* infections have been shown to impair cognitive ability and even manipulate rodents to lose their fear of felines. Delusions, hallucinations, and other psychiatric symptoms have also been observed in acutely infected humans. Serotyping for *Toxoplasma* antibodies in patients diagnosed with schizophrenia has generated positive results, and youths that are exposed to cats during their childhood have an increased risk of becoming schizophrenic (41).

Studies are continuing to show a stronger correlation between the acquisition of toxoplasmosis and the development of schizophrenia, but what could this mean for those currently suffering from schizophrenia?

An entirely new perspective on treatment of this disease would emerge within the medical field. Even more importantly, prevention of this mental disorder could become a realistic notion, leading to the reduction in the amount of people that are diagnosed with schizophrenia on an annual basis. This concept is revolutionary and potentially life-changing for millions of people worldwide.

This literature review will discuss the diseases toxoplasmosis and schizophrenia in detail before examining the growing correlation of the acquisition of schizophrenia after infection by *Toxoplasma*. An emphasis will be placed on the mechanisms this parasite uses to invade the host so as to provide a better basis of understanding for the reader and possibly present a premise on how to prevent initial infection.

SCHIZOPHRENIA

Definition and History

Schizophrenia is a psychiatric disease that results in altered behavior, emotions, and thinking (8). The disease is commonly characterized by difficulty to recognize what is real and not real, function normally in social settings, experience standard emotional responses, and think logically (1).

Schizophrenia was only recently recognized as an explicit disease in the medical field (1). This condition was first described and labeled in 1860 by Bénédict Morel after observing an intellectual deterioration in a young adult (17). It was not until 1911 that the term schizophrenia was established by a Swiss psychiatrist, Eugen Bleuler, to describe people that he noted suffered from an identifiable psychotic disorder. He used this term to brand patients with mild symptoms that would not be considered schizophrenic by current standards (17). Schizophrenia thus became a prevalent diagnosis for troubled patients, especially in Switzerland where it still remains a popular diagnosis to this day (17). Those suffering with schizophrenia were forcibly institutionalized for a majority of their adult lives. This practice was standard until the late 1950s when increased knowledge of the nature of schizophrenia helped reveal more beneficial therapies to treat this disease (46).

Onset and Prevalence

The heritability rate of schizophrenia is thought to be greater than eighty percent, suggesting genetics play a significant role in the occurrence of this disease (1, 8). Onset of schizophrenia most commonly arises in individuals roughly between fifteen and thirty-five years of age (8, 45). This disease rarely develops in children, whom represent less than one percent of diagnosed patients, and the elderly (8).

Schizophrenia affects approximately twenty four million people worldwide and one percent of the human population within the United States and Europe (7, 12, 41, 45). The lifetime frequency of this disease across the world is estimated to be four out of every thousand people (4). Of one thousand adults, seven are expected to suffer from this debilitating disease (45). Although schizophrenia affects an equal amount of both men and women, it tends to develop later and less severely in women for unknown reasons (1, 4). Higher rates of schizophrenia are found in migrant and homeless populations (4).

Surprisingly, the prevalence of this disease is lower in developing countries than that of industrialized nations. Statistics, however, are

highly complicated due to varying types of schizophrenia affecting different areas in contrasting proportions (4). Catatonia, a form of schizophrenia where individuals enter a stuporous state for a long period of time, represent ten percent of cases in developing countries compared to one percent in developed (4). Hebephrenia, schizophrenia leading to a dramatic loss of personality, presented itself in thirteen percent of cases in industrialized countries but only four percent in developing areas (4). Fifty percent of all individuals that display the traits of schizophrenia are not being treated for the disease, ninety percent of which are in developing nations (45).

Symptoms and Diagnosis

Symptoms of schizophrenia are classified as either positive or negative. Positive symptoms are those that are not experienced by people in the normal population, such as delusions, hallucinations, and thought disorder (1, 3, 8, 17). Negative symptoms are common thoughts, feelings, or behaviors that are diminished or absent in a schizophrenic individuals, such as speech deficiency, reduced emotional communication, lack of motivation, anhedonia (reduced ability to feel pleasure), and disorganization of behavior and emotions. Other indicators of this disease are depression, anxiety, irritability, agitation, and sleep deprivation (1, 3, 8, 17). Schizophrenia also causes several cognitive impairments, including changes in memory, judgment, attention, association, and perception (1, 3, 8, 17).

Not all individuals experience the same combination or severity of symptoms, so an array of diagnostic tools are used to identify schizophrenia in patients. Clinical diagnosis of schizophrenia is based on symptoms suffered by the patient and the progression of the illness over time (1, 8). Blood tests and brains scans help to rule out other conditions with similar symptoms (1).

Identification of schizophrenia is highly reliable when standard diagnostic criteria are combined with direct observation and comprehensive history (1, 8). Information is collected from the patient, known associates, and close relatives. Doctors will inquire about the length of symptoms experienced, changes in ability to function, family history, developmental background, medical conditions, substance abuse, and responsiveness to prescribed medications to properly diagnose patients (1, 8).

Treatment and Prognosis

Those diagnosed with schizophrenia are placed on a life-long prescription of antipsychotic medications that alter the balance of chemicals in the brain in an attempt to alleviate symptoms (1). Common side effects are sedation, weight gain, restlessness, dizziness, and tremors (1). Antipsychotics can also lead to the development of tardive dyskinesia, a condition characterized by uncontrollable movements. Other medications may be considered and tried if use of antipsychotics results in little or no success (1). However, therapy and support services can help patients suffering from schizophrenia cope with symptoms and side effects from medications (1, 17).

Prognosis is unpredictable and varies from person to person. Symptoms usually improve with continued use of medication and will almost always return if the individual chooses to stop drug usage (1). A combination of increased knowledge about schizophrenia and improved treatment options has helped

to lower the lifetime morbid risk of patients with this disease to one percent (8). Despite this, physicians are always searching for a superior treatment solution to help relieve those twenty-four million people suffering from schizophrenia across the world.

TOXOPLASMA GONDII

Definition and History

Toxoplasma gondii is a single-celled, apicomplexan parasite capable of infecting nearly all nucleated cells within a vertebrate host displaying both phagocytic and non-

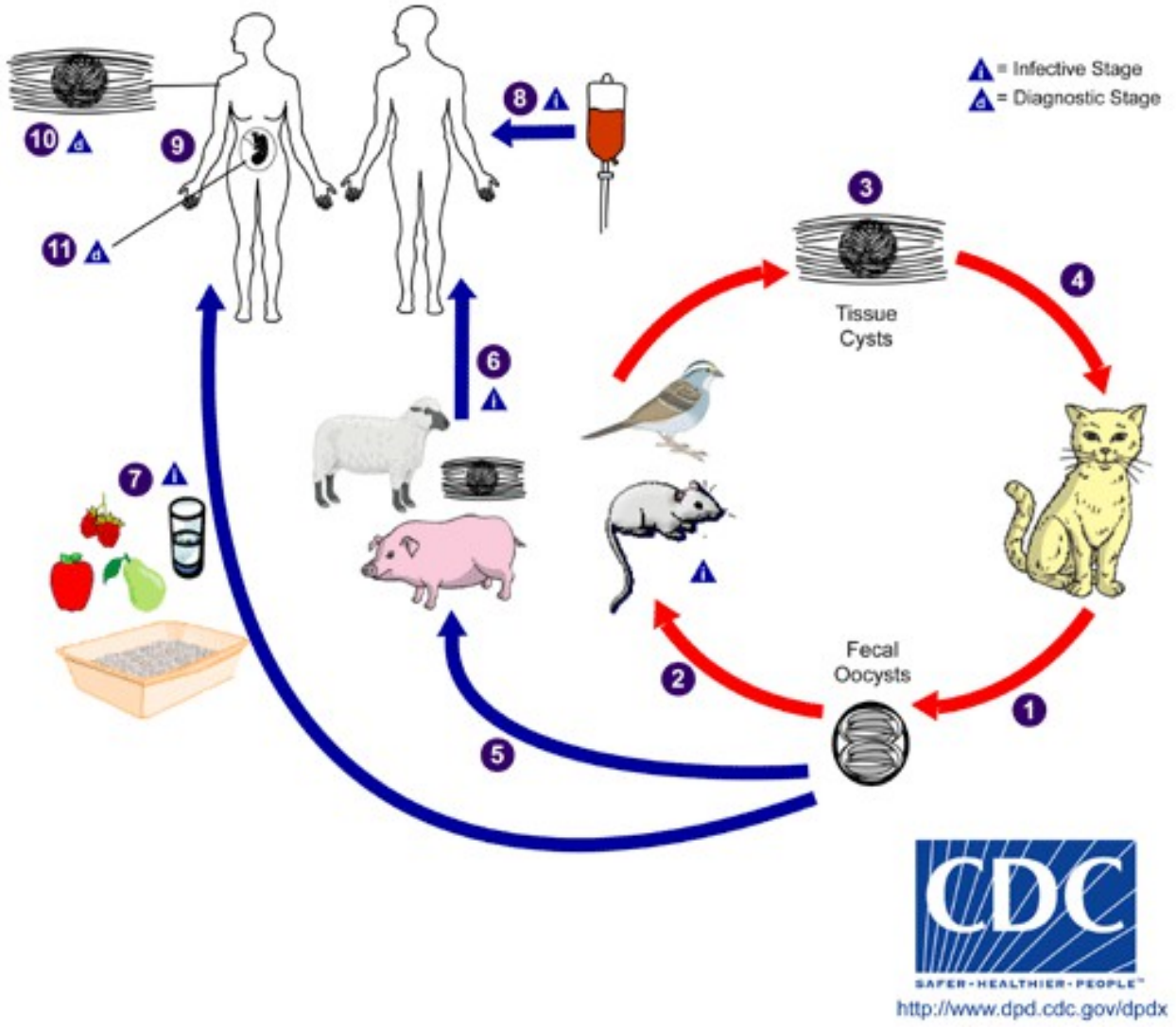

Figure 1: Diagram of the life cycle and transmission of *Toxoplasma.* This image was taken from the Centers for Disease Control and Prevention, 2013 (6).

phagocytic properties (5, 6, 14, 15, 29, 33, 34, 42). Cats are the only definitive host with the infection of other species being purely accidental (37, 6).

Toxoplasma was first identified in mice by Charles Nicolle and Louis Manceaux in 1908. Toxoplasmosis was later acknowledged as an infectious disease in 1932 after causing the

congenital infection of an infant (27). This disease was recognized as severe and potentially fatal in 1968 after several cases of toxoplasma encephalitis in patients suffering from hematological malignancies (27). *Toxoplasma* was later noted in 1983 as being extremely dangerous for immunocompromised individuals due to uncontrolled replication of the parasite (10, 27).

Prevalence

Toxoplasma is arguably the most successful protozoan parasite on Earth (13, 16). It is estimated that fifty percent of the population worldwide and nearly one hundred percent of people within some local populations are seropositive (33, 37). Practically fifty percent of all immunocompromised individuals suffer from the devastating effects of chronic toxoplasmosis (10, 42).

Life Cycle and Transmission

Toxoplasma starts as oocysts in cat feces that sporulate in the environment within one to five days (6, 37). These oocysts can either re-infect another feline or enter an accidental host species, such as humans or rodents (See Figure 1). Shortly after ingestion, oocysts transform into the infective tachyzoites and undergo endodyogeny (asexual reproduction) within the intermediate host (6, 16, 23, 37). Infected cells rupture within six to eight hours and release an astounding range of sixty-four to one hundred twenty-eight parasites (10, 23). After liberation, parasites spread throughout the host body via lymphatics and the blood stream (23). Acute infection is commonly associated with inflammation and necrosis caused by the vast distribution of these harmful organisms (10).

Within accidental hosts, *Toxoplasma* then enters neural and muscle tissue to form encysted bradyzoites (6, 23, 37). Bradyzoites appear within a short six to eight days but last a lifetime (23). This is usually unproblematic in a healthy host because the parasites are sequestered by a strong immunological response (6, 10, 37). A low level of cyst reactivation is kept in check by the immune system, but lowered immunity can result in the recurrence of tachyzoites (6, 10, 37).

Depending on if the accidental host species is a feline pray animal, bradyzoites may be ingested by a cat and re-released into the environment as unsporulated oocysts (6, 37). Both merogony and gamogony (sexual reproduction) occur within the intestines of the feline species (10, 16, 23, 37). The cat is then capable of excreting over one hundred million parasites in a single day if acutely infected (23).

There are four modes of *Toxoplasma* transmission known in humans. Foodborne transmission occurs when an individual eats the flesh of an infected animal, commonly contaminated pork product (6, 37). Zoonotic transmission is caused by being in close proximity with infected cats (6, 37). People can easily ingest oocysts from contact with feline excrement, whether in a public market or a cat-containing household. Congenital transmission is that which is passed from mother to offspring before birth (6, 23, 37). *Toxoplasma* is well known for penetration of the placental barrier placed between it and the fetus, leading to damaging repercussions for the helpless infant. The last mode of transmission occurs via blood donation or organ transplantation (6, 37). Parasites can be unknowingly present in tissue or blood provided to a sickly patient, leading to subsequent infection.

Parasitic Host Invasion: The Basics

One of the most astounding observations made concerning *Toxoplasma gondii* is its remarkable capability of invading a vast number of warm-blooded, vertebrate hosts. This process begins with the entrance of the parasite into any nucleated cell, including both phagocytic and non-phagocytic (5, 14, 15, 29, 33, 34, 42). The presence of a variety of general receptors on the outer surface of parasite allows parasitic attachment to nearly all cell varieties (33, 37). Internalization of *Toxoplasma* occurs via one of two separate mechanisms: engulfment or active invasion (29, 42).

Phagocytosis of the parasite commonly results from a specialized cell, such as a macrophage, coming into contact with the parasite at any location other than the parasite's apical end (42). The anterior end of the parasite contains the organelles that secrete compounds used for active invasion, all of which are unnecessary when the host cell phagocytizes the invader. This process usually successfully eliminates pathogenic antigen from the host via degradation of the internalized parasite. This process triggers the lysosome, a specialized vacuole containing acidic compounds, to fuse with the phagosome enclosing the foreign invader. *Toxoplasma* is specially equipped to prevent phagosomal-lysosomal fusion, and thus avoid internal acidification (13, 33, 34, 37). The parasite is then free to multiply without constraints via endodyogeny, or binary fission, within the phagosome of the host cell (13, 23, 37, 42).

Active invasion is a much more complex process of phagocytosis, requiring cooperation of the parasite and the host cell. Although the specific steps are not entirely known, the results of multiple studies suggest a mechanism for active invasion. The first and arguably most important step in this process is the initial attachment of the parasite's apical pole to the host cell cytoplasmic membrane (37, 42). The conoid, a specialized organelle of *Toxoplasma,* is pushed out of the parasite into the environment where it indents the host cell membrane. The disturbed cell responds by excreting amorphous protrusions, or pseudopods. Upon contact with the parasite, these create flexible junctions to stabilize the interaction between the two cells (23, 42). This action triggers the release of penetration-enhancing factor (PEF) by the rhoptries of the parasite, a highly important enzymatic compound (described in greater detail below). PEF alters the viscosity of the host cell membrane to allow for invasion as well as aids in the creation of the intracellular compartment in which the parasite will continue its life cycle (5, 13, 29, 30, 37, 42). Once fully internalized, the invagination in the target cell is closed and the newly-formed parasitophorous vacuole (PV) is altered in order to sustain the parasitic invader.

Active invasion, as suggested by its name, requires an input of energy by both of the involved cells. This allows penetration to occur in less than one quarter of the time required for phagocytosis (14, 23, 31, 42). Glucose availability to the parasite greatly affects the infection rate. Moreover, high glucose concentrations counteract prior treatment with respiration-blocking cyanide (42). This demonstrates that the parasite is dependent on respiration via oxidative phosphorylation for energy production unless an outside energy source, such as glucose, is provided. The high concentrations of glucose allow for substrate-level phosphorylation to produce enough energy for invasion despite

the fact that it is considerably less efficient than completing the entire process of respiration. On the other hand, internalization of *Toxoplasma* also requires host cell energy commitment. *Toxoplasma* has been shown incapable of invading energy-depleted cells, such as embryotic chicken erythrocytes, in experimental settings (42). The restoration of ATP in the environment reestablishes invasion competence.

Consecutive exocytosis of the contents of the apical mirconemes, rhoptries, and dense granules mediates host cell infiltration by *Toxoplasma gondii* (13, 30, 37). The compounds found within these organelles are regulated by both the physical separation of the excretion sites on the parasite's plasma membrane and the differentiation of signaling mechanisms (13). The mirconemes are primarily involved in parasitic attachment and eventual penetration. As described earlier, the rhoptries secrete PEF to disrupt the target cell membrane, create moving junctions, and aid in the development of the parasitophorous vacuole (5, 13, 29, 30, 37, 42). Dense granules are active throughout the stages of *Toxoplasma,* secreting proteins during a majority of the parasitic life cycle (13).

Of these three organelles, the rhoptries are considered to be the most important vis-à-vis host cell invasion, containing over an astounding thirty proteins (30). Unlike most semi-spheroidal secretory organelles, the rhoptries resemble a club with a rounded end and extending neck. They are the only acidic structures found within *Toxoplasma gondii* (13, 37). With an average of roughly ten rhoptries per cell, these organelles can occupy up to an astonishing thirty percent of the total cell volume (13). The conversion of immature to mature rhoptries is characterized by the subcompartmentalization of the ROP proteins in the bulbous base and the RON compounds in the prolonged duct (13). The RON4 protein relocates to the moving junctions of the host cell membrane during invasion to aid in the formation of the connecting structures between these two interacting cells (13). The combination of RON2, RON4, and RON5 forms a protein combination (Ts4705) that associates itself with the adhesion protein TgAMA1 derived from the mirconemes (13). These newly formed moving junctions then organize parasite and host cell surface components, determining what to selectively integrate into the parasitophorous vacuole, such as the Ts4705-TgAMA1 compound described above. The exclusion of many host cell proteins in the membrane of the parasitophorous vacuole prevents the fusion of the lysosome to this compartment (13, 37). The ROP proteins have been exclusively studied and will be described in further detail below.

A majority of *Toxoplasma* isolates found within Europe and the United States belong to one of three strains, all varying in virulence capabilities when tested on experimental mice (13, 15). Mice infected with the Type I strain died within a very short time, usually between six to ten days (13). Test subjects exposed to the Type II and III strains almost always survived initial infection but remained chronically diseased and highly seropositive throughout the remainder of their life (13, 15). Forward genetics have demonstrated that the main differences between the varying strains and their virulence proficiencies are mediated by the ROP18 protein kinase (13, 15). The Type I and II alleles of ROP18 differ in both nucleotide sequence and expression levels than the gene encoding ROP18 found

within the Type III allele (13, 15). The specific functions of this protein kinase are discussed below.

Parasitic Host Invasion: Gliding Motility For Movement And Penetration

Lacking both flagella and cilia, more commonly understood structures for transportation, *Toxoplasma gondii* and other Apicomplexan parasites utilize a specialized form of substrate-dependent motility known as gliding to traverse solid surfaces (14, 35). Gliding motility is reliant on the actin filaments of only the parasitic cytoskeleton and involves a highly preserved group of secretory adhesions (14, 35, 42). Unlike crawling motility, cell morphology is unaltered and pseudopod extension is unnecessary (35). Parasitic gliding is an energy-dependent process that can move the cell at rates of up to ten micrometers a second and helps to explain the incredible speed of active invasion (35, 42). The most common method to test for the presence of gliding locomotion is to stain the surface compounds, such as SAG1, of the parasites that employ it (35, 42). These components are deposited on the solid surfaces being traversed, allowing for any movement to be easily tracked as a function of time (See Figure 2).

Cytochalasins and other chemical agents are used to inhibit both motility and active invasion, demonstrating the importance of actin filaments for transportation (14, 35). These compounds also poison the process by which surface particles are translocated from the anterior to the posterior end of *Toxoplasma* and other related parasites (35). The effect of cytochalasins on the movement of the parasitic cells is measured by the same staining method described above. Due to the fact that cell replication does not occur outside of the target host cell, cytochalasin treatment does not disrupt parasitic division (14). Two separate mutations are known to confer cytochalasin resistance in *Toxoplasma* cells (14, 35). These mutations map to the *act1* gene and alter the area of the unnamed actin monomer that defines cytochalasin binding (14). On the protein level, they mutate two interacting alpha helices that modify the specific regions Val-139-Met and Ala-295-Asp (14).

The entire system works similar to a well-oiled machine, involving the cooperation of capping membrane elements and the actin myosin motor (See Figure 3). Capping is the transfer of protein components from the apical to the posterior end of the parasitic membrane, where they are then shed to create a parasitic trail (35). Gliding motility results from the interaction of these proteins with fixed compounds on the solid surface, pushing the parasite forward with every relocation of a membrane-bound component (35). The actin filaments of the myosin motor create a fixed platform on which myosin moves (5, 14, 33, 35). The myosin tails are thought to indirectly act with the receptors of the extracellular substrate via TRAP-like proteins, resulting in the gliding motion of *Toxoplasma gondii* (35).

Thrombospondin-related adhesive protein (TRAP) is a highly important component of invasion by malarial sporozoites associated with binding to target cell heparin sulfate proteoglycans (35). Within a region of the thrombospondin type I motif found in TRAP is a specific integrin associated with cell-cell interactions, commonly used for parasitic homing to the hepatocytes of the liver (35).

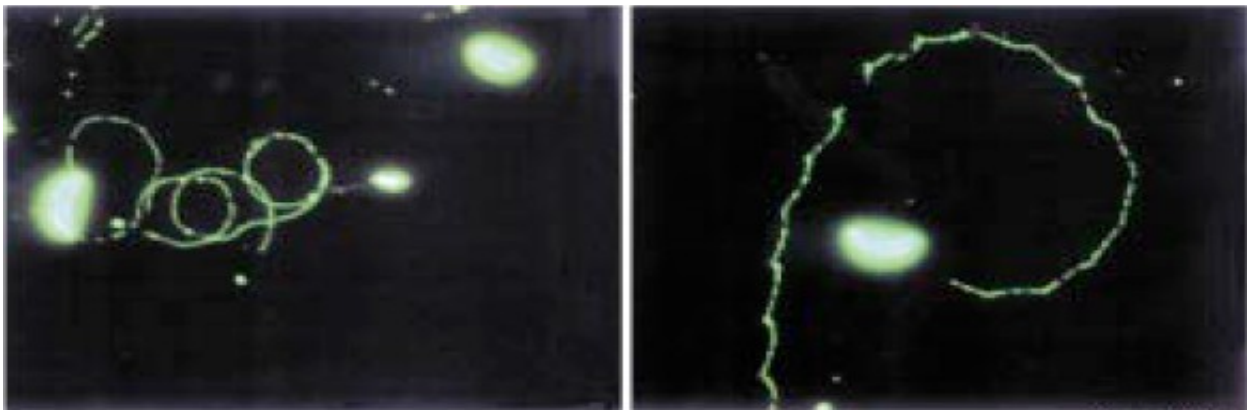

Figure 2: Fluoresent staining of the *Toxoplasma* surface protein SAG1 by monoclonal antibodies. This image was taken from Sibley, Hakånsson, and Carruthers, 1998 (35).

Null mutations within a TRAP gene have been engineered and shown to produce proteins incapable of migration or invasion in either mosquitos or rodents. The same results were obtained when TRAP was completely removed from malarial cells (35). TRAP-like proteins expressed by *Toxoplasma* and other Apicomplexan parasites, such as MIC2, are secreted onto the target cell surface. MIC2 proteins then bind to the membrane receptors of the host cell where they are capped by myosin to allow for gliding motility (35).

Parasitic Host Invasion: Penetration-Enhancing Factor (PEF)

Penetration-enhancing factor (PEF) is secreted by the rhoptries and most likely alters the morphology of the host cell, allowing for a greater infection rate (23, 24, 28, 30, 31, 43). The initial location of PEF has been determined by synthesizing a fluorescent, monoclonal antiserum. Results show the emission of light in the apical end of *Toxoplasma* cells in the exact location and morphology of the rhoptry organelles (31). Although these antibodies were able to decrease the efficiency of infection by roughly five hundred percent, they were not able to completely prevent parasitic penetration (23, 31, 43). This implies that either PEF is not the sole determinant of infection by *Toxoplasma* or the monoclonal antibody has a low affinity for the antigen. However, the fact that these monoclonal antibodies were able to neutralize PEF activity and lower infection rates at any degree has lead scientists to theorize that this protein resembles an enzyme with an active site, not a membrane-destabilizing detergent (28, 31, 43). There is currently no proof of this assumption though.

PEF has been shown in vitro to be temperature-dependent. Parasites exposed to an environment that increased gradually in temperature to that of the human body showed a dramatic proliferation in the rate of PEF release (43). These results may indicate that the parasite is signaled to penetrate cells once inside the host body via the environmental temperature. This would prevent unnecessary use of energy and synthesized compounds until the appropriate time.

In vitro, the concentration of PEF in the environment has been shown to be directly

proportional to the number of damaged host cells (23, 31, 43). At very minute concentrations, PEF does not induce morphological change in exposed cells, demonstrating that a threshold concentration must be met for effective invasion (23, 31). The presence of PEF in amounts exceeding that minimum concentration requirement increase invasion of *Toxoplasma* by an astonishing one thousand percent in experimental settings (23).

Although the exact chemical structure of this protein is unknown, several studies have suggested that PEF resembles polycationic peptides, unusually high concentrations of which are found in the rhoptries (43). Experiments were conducted to analyze the infection rates of cells exposed to differing

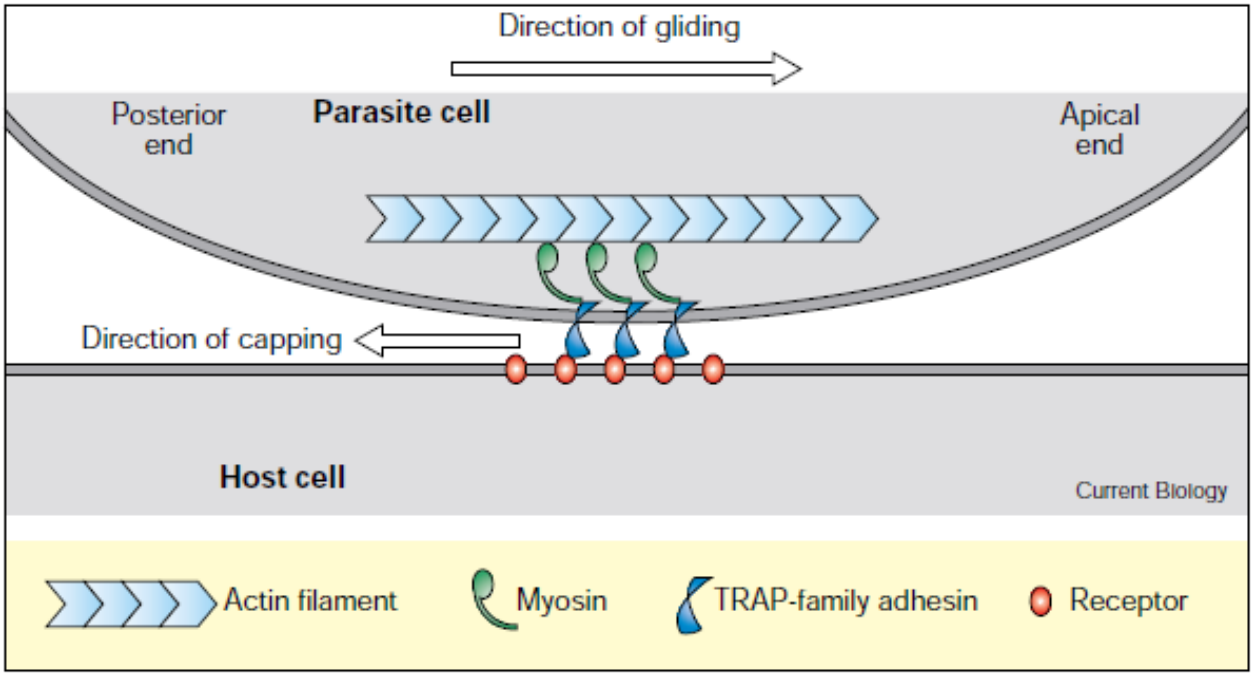

Figure 3: Molecular model of the mechanisms used to drive gliding motility of *Toxoplasma gondii* and other Apicomplexan parasites. This image was taken from Sibley, Hakånsson, and Carruthers, 1998 (35).

concentrations of three different polycationic peptides: polylysine, polyhistidine, and polyarginine (43). Due to the likely similarity of these compounds to PEF, results were considered relevant to the function of the more mysterious PEF protein.

All three polycationic peptides roughly doubled the infection rate of *Toxoplasma,* but polyhistidine induced the least amount of structural damage to host cells (43). Polylysine, on the other hand, appeared to most dramatically affect the target cells, inducing the highest rate of infection and the

demonstrating the most severe morphological destruction to the target cells (43). At exceptionally high concentrations, these peptides even began to strip the host pellicle, partially condense the host chromatin, and damage the parasite itself (43). In the natural

environment, these extreme concentrations are not expected to exist due to the detrimental effects on *Toxoplasma* cells. Although these three polycationic peptides are not perfect representations of PEF, they provide a model of the possible function of this compound. Future studies that could bind PEF monoclonal antibodies to these polycationic peptides would further support the relatedness of these two proteins.

Parasitic Host Invasion: Importance of the Parasitophorous Vacuole (PV)

The parasitophorous vacuole (PV) is a highly specialized structure that allows for the reproduction of *Toxoplasma gondii* without host cell interference (13, 15, 34). This synthesized niche serves a variety of functions for the parasite, including the import of nutrients, export of waste, and communication with the host cell (13). Fluorescent labeling of host membrane lipids (DiIC16) and proteins (DTAF) also demonstrated that target cell surface components are integrated into the PV during formation (23, 29, 34). However, the PV also excludes or contains a lower number of certain membrane components, such as integral membrane proteins, than the target cell (29). This validates the selectivity function attributed to the temporary moving junctions during PV formation (13, 29, 37).

The structure of the PV is thought to be modified via an I-63 reactive antigen and other relatives most likely found in PEF (24). This hybrid membrane also contains proteins secreted by the parasite itself, a contribution which prevents recognition as either a foreign compartment or a host phagosome (24, 33, 34, 36, 37). Upon formation, the PV is rapidly surrounded by host cell mitochondria and endoplasmic reticulum to further discourage phagolysosome creation (33). Normal phagosomes undergo two acidification processes: a primary respiratory burst via the activity of NADPH-oxidase and a secondary acidification due to lysosomal fusion (36). The disguised PV is able to avoid both of these happenings in order to protect the susceptible parasite inside (24, 33, 34, 36, 37).

Several studies have been conducted to test the effects of pH on *Toxoplasma* cells in order to evaluate the importance of lysosomal evasion. The measured viability of incubated parasites grown in media of pH 6.0 were reduced by upwards of sixty percent (34). This is considered only slightly acidic, demonstrating that *Toxoplasma gondii* is highly affected by environmental pH. Fortunately, immunocompetent host cells are able to overcome this imposing barrier. In order to terminate the invading parasites, the activation of mononuclear phagocytes is still possible via the actions of lymphokines and monokines (34).

Parasitic Host Invasion: The ROP2 Protein Family

The ROP2 protein family, produced by the rhoptry organelles of *Toxoplasma,* is distinguished by several different physical characteristics. The N-termini of these proteins contain arginine-rich stretches, and the C-termini include primarily a hydrophobic sequence that traverses the phospholipid membrane (13). The genetic makeup of the molecule is also characterized by the presence of a high density of proline residues and charged amino acids (13). During the initial invasion of the host cell, the N-termini of the ROP2 proteins interact with target cell mitochondria to associate them with the parasitophorous vacuole (5, 13). As discussed earlier, this provides protection for the

Toxoplasma parasite by preventing phagosome-lysosome fusion (33).

The ROP2 protein complexes are assembled in the endoplasmic reticulum (13). Once released, the undeveloped proproteins are placed within coated vesicles that translocate to the Golgi apparatus. Aided by particular sorting signals, immature ROP2 proteins are placed within their individualized immature rhoptries (13). In this location, the N-termini of the proproteins are cleaved between amino acid residues thirty-three and thirty-four (13). This results in fully-developed and functional ROP2 proteins, most likely because the N-termini contain the signal sequences required for protein development.

The deletion or inactivation of ROP2 proteins resulted in a multitude of devastating effects for the invading parasite (5, 13). Rhoptry biogenesis and cytokinesis are heavily impaired, and the invasion and replication of parasitic cells are drastically reduced. Uptake by the host cell is greatly diminished and the association of the mitochondria with the PV could not be detected (5, 13). These observations support that the ROP2 protein family is a necessity for the virulence of *Toxoplasma gondii* and other related Apicomplexan parasites.

The ROP2 family includes the ROP2, ROP3, ROP8, and the newly discovered ROP4 proteins, initially identified by their interactions with cross-reacting monoclonal antibodies (5). The recently uncharacterized ROP4 protein is secreted by the rhoptry organelles either during or shortly following invasion of the parasite and subsequent association with the PV (5). Host cell factors then target ROP4 for phosphorylation in the infected target cell by either one of the available parasitic or host cell kinases (5).

Parasitic Host Invasion: Other ROP Proteins

Although the ROP2 protein family is the primary focus for experimental studies, other ROP proteins exist within *Toxoplasma gondii.* Initially thought to be of similar importance as ROP2, ROP1 has been proven by successive gene knockouts to be unessential for either parasitic penetration or intracellular survival (5). ROP1 contains two extremely charged regions, one being highly acidic and the other being exceptionally basic (23). This physical phenomenon is currently unexplained. Due to the determination that ROP1 is also never cleaved in the same fashion as the proteins of the ROP2 family, ROP1 may instead be involved in protein folding or functionality (5). No substantial evidence currently exists for this hypothesis. Despite the fact that its specific functions remains unknown and relatively unimportant, ROP1 protein has proven to be a useful model for following protein trafficking and processing (5).

ROP18 protein kinase, as discussed earlier, is the major mediation factor in the determination of the three significant strains of *Toxoplasma gondii* (13, 15). This was demonstrated by identifying virulence loci, one which encoded the ROP18 protein. To

ensure that the differing ROP18 proteins are indeed what determine infectiousness, a proposed study could remove and transfer ROP18-encoding genes between the three *Toxoplasma* strains. If pathogenicity was affected, ROP18 is almost certainly responsible for virulence.

Upon invasion of the host cell, ROP18 proteins migrate from the interior of the rhoptries to the membrane of the PV in order to phosphorylate two parasitic components not yet identified (13). When the ROP18 gene was overexpressed, the rate of parasitic reproduction increased drastically. Induced subsequent mutations within those same ROP18 proteins devastated *Toxoplasma* and decreased both enzymatic activity and parasitic growth capabilities (13). This is due to the fact that ROP18 is responsible for phosphorylating immunity-related GTPases (IGRs) that block the recruitment of host cell protein Irgb6 (15). Not surprisingly, studies have proven that Irgb6 is a requirement for parasitic destruction. Expression of ROP18 is both essential and adequate for inhibiting the recruitment of Irgb6 (15).

ROP16 is also capable of kinase activity, unlike the ROP2 protein family that contains modern molecular fossils unable to transfer phosphate groups between molecules (13). In contrast to ROP18, ROP16 is translocated from the rhoptry organelles to the target cell nucleus (13). Once internalized by the nucleus, this protein kinase is able to decrease the production of IL-12 and its subsequent secretion (13). This aids in the protection of the invading parasitic cell especially in *Toxoplasma* Type I and III strains.

Symptoms and Diagnosis

Common symptoms of infection by *Toxoplasma* are swollen lymph nodes, migraines, and muscle pain for a prolonged period of time (6, 37). Ocular toxoplasmosis results in reduced vision, increased eye sensitivity, blurred vision, redness of the eye, and uncontrollable tearing (6, 37). Acute infection can also cause severe eye and brain damage, abnormal changes in head size, deafness, seizures, and cerebral palsy in newborn infants (6, 37, 41).

The diagnosis of this disease begins with the detection of *Toxoplasma*-specific antibodies, IgG, IgM, and IgA, via a Sabin-Feldman dye, indirect fluorescent antibody (IFA), or enzyme immunoassay (ELISA or immunoblots) test (6). The presence of IgM represents a recent infection while past exposure is characterized by the test positivity for IgG antibodies (37). A polymerase chain reaction (PCR) test on amniotic fluid after eighteen weeks of gestation can determine if a fetus is infected (6). If the patient exhibits a chronic infection, an MRI or CT scan may be required to obtain more information on the severity of the disease (37).

Immunity

Immunity to *Toxoplasma* depends on a strong humoral and cell-mediated response by the infected individual. Despite both responses being necessary, cell-mediated immunity is considered to be of particular importance when fighting this parasitic invader (37). The goal of the body is to convert the infective tachyzoites to dormant bradyzoites in order to reduce internal damage (32, 37). IL-12 stimulates CD4 T cells and natural killer cells to produce the cytokine IFN-γ which is used in innate defense and adaptive immunity (10, 32, 34). Some cells require an IFN-γ primer to

produce significant levels of IL-12 to start the defensive cascade (10, 32, 34).

IFN-γ serves multiple purposes in immunity. This interferon promotes conditions within the body that favor its production. As the number of IFN-γ increases, it becomes parasiticidal to *Toxoplasma* by inducing the host degradation of tryptophan, an amino acid needed for the growth and reproduction of parasitic tachyzoites (10). Without this protein, the infective parasites starve. IFN-γ also activates macrophages that prevent the production of H-uracil, a compound required by the parasite but not the phagocyte, inhibiting tachyzoite replication (10). In return, these activated macrophages release nitric oxide antagonists that block the inhibition of IFN-γ (10). Thus, the cycle of immunity continues.

Several studies have shown that CD8 cells seem to be of more importance that CD4 when fighting this parasite, resulting in the formation of two hypotheses to explain this phenomenon. Hypothesis one proposes that CD8 cells are more frequently activated due to the fact that a majority of bodily cells express MHC class I molecules (10). Hypo-thesis two insists that CD8 cells are more valuable when fighting infection because they are cytotoxic and automatically kill the parasitic invader (10). Both postulates make very strong arguments.

Treatment and Prognosis

Most people of good health infected with *Toxoplasma* recover without any form of treatment (6, 37). For those that require assistive medication, sulfadiazine is often used in combination with pyrimethamine (6, 11, 37). If the patient is allergic to sulfa drugs, sulfadiazine is replaced with clindamycin, atovaquone, clarithromycin, azithromycin, or dapsone. Leucovorin (folonic acid) is also prescribed to protect the bone marrow from the toxic effects of pyrimethamine (6, 37).

AIDS patients are placed on antiretroviral drugs, if not already, to increase CD4 count and the functioning of their immune system (11, 37). In acutely-infected pregnant women, spiramycin is prescribed for the first and early second trimester. For the remainder of the pregnancy, women are medicated with sulfadiazine, pyrimethamine, and leucovorin (6). After birth, infants tested seropositive for *Toxoplasma* remain on a strict regime of these three drugs for at least the first year of life to prevent further parasitic damage (6).

As mentioned above, a majority of immuno-competent individuals recover from toxo-plasmosis without treatment. Infants infected within the first trimester have a poor prognosis due to likeliness of extensive tissue damage (6, 23, 37). Infiltration of the placenta by *Toxoplasma* during the first initial months of pregnancy usually results in death, devastating physical limitations, or severe mental problems for the fetus. For the immunocompromised, such as HIV patients, lifelong treatment is mandatory. Prognosis for these individuals is highly dependent on diagnostic timing, rate of infection, and response to treatment (6, 37).

THE CONNECTION

Effect of *Toxoplasma* on Rodent Mentality

Studies have demonstrated that the learning capacity of mice is dramatically stunted by *Toxoplasma* infection and decreases as the number of parasitic cysts form in the brain (44). Rats' natural aversion to the scent of cats is also diminished, suggesting that

chemicals within the host brain are somehow altered (2). This phenomenon not only leads to the increased predation rate of *Toxoplasma*-infected rodents by cats, but also allows the parasite to complete its life cycle within the feline host (2, 41, 44).

Association between *Toxoplasma gondii* and Schizophrenia

Patients diagnosed with schizophrenia are more likely to simultaneously be infected with *Toxoplasma* than other subsets of the population (7, 9, 19, 21, 26, 38, 39, 40, 41). *Toxoplasma* has been shown to alter neurological behavior and transmission, especially in those acutely infected, that resemble schizophrenia (9, 41). Those suffering from a heavy parasitic load can also experience delusions, hallucinations, and other psychiatric symptoms (9, 41). These symptoms are more commonly experienced in those who are immunocompromised (21, 41). Over sixty percent of individuals suffering from both AIDS and toxoplasmosis suffer from an altered mental state (21). Serologically positive individuals with an asymptomatic infection of *Toxoplasma* also display a lower psychotic stability under pressure than those never exposed to the parasite (18).

Other studies have found that childhood exposure to cats can increase the risk of developing schizophrenia later in life (39, 40, 41). Although this is not direct evidence of the relationship between the two diseases, it does pose an interesting point. The more contact an individual has to felines, the more likely they are to be infected by this prevalent parasite. Antipsychotic drugs used to treat this mental disorder and related diseases also inhibit the development of *Toxoplasma* grown in cell cultures (22). This suggests that psychotic symptoms may decrease with medication because of its parasiticidal effect on *Toxoplasma.*

Areas such as France and rural Ireland have a substantially high amount of people diagnosed with schizophrenia on an annual basis, a rate that coordinates eerily with the contraction of this parasite (41). Schizophrenics simultaneously infected with *Toxoplasma*, despite parasitic latency, also have an increased mortality rate of nearly five times the amount of those not tested seropositive (12). Whether toxoplasmosis results in schizophrenia or not, the two diseases undeniably blend with one another in a devastating way for the patient infected.

Major Studies

Multiple studies within the scientific community appeared in the latter half of the twentieth century concerning the relationship between toxoplasmosis and schizophrenia, some of which will be discussed below. This correlation has been investigated for numerous decades but has just recently become more widely publicized, bringing to light masses of data results collected in past years.

In 1953, a Polish study reported twenty-five percent of nearly seven hundred controls were seropositive for *Toxoplasma* compared to fifty-two percent of nearly one thousand psychiatric patients (41). An experiment in Mexico that spanned from 1953 to 1965, a total of twelve years, confirmed the presence of antibodies for *Toxoplasma* in eighty-six percent of nearly one thousand psychotic patients. In contrast, only thirty percent of people from the general population tested positive for this parasitic invader (41).

A rather extensive study in 1999 consisted of pregnant women, shortly before giving birth, donating sera that was immediately tested for *Toxoplasma* IgM and IgG class antibodies. Results were later compared to the rate of children that developed diagnosable schizophrenia in adolescence or adulthood (41). Women experiencing an active infection, in order words producing large amounts of IgM antibodies, more commonly gave birth to children that later became schizophrenic (12, 26, 41). IgM antibodies were not found for rubella virus or cytomegalovirus, two other common perinatal pathogens, eliminating the possibility of either leading to the acquisition of a psychotic disorder later in life (41).

Experiments concerning the relationship between *Toxoplasma* and schizophrenia were not only limited to the twentieth century. In 2003, a German study separated first-episode schizophrenics by the amount of antipsychotic treatment they received in the past. The antibody levels for *Toxoplasma* correlated with the amount of antipsychotic medications received, supporting the idea that drugs used to treat schizophrenia lower the efficacy of the parasite (41). The results were consistent for both blood sera and cerebrospinal fluid. In a 2007 study, sixty-six percent of patients diagnosed with schizo-phrenia were also positive for IgG titers (7, 26). This percentage was nearly three times higher than volunteers whom were either perfectly healthy or suffering from depressive disorder, suggesting this parasitic infection relates solely to psychotic maladies (7). A couple of years ago, gray matter density within the brain of schizophrenic patients was found to be significantly less when also suffering from a latent infection of *Toxo-plasma* (20).

A recent meta-analysis of forty-two studies conducted in seventeen countries over the course of five decades produced an odds ratio of 2.73 (38). Despite this number being only moderately convincing, it exceeds that of any environmental or genetic factors identified (12, 38). Extensive evidence such as this should be seriously acknowledged, providing an explanation and even a treatment for those suffering from debilitating psychotic diseases.

CONCLUDING IMPLICATIONS

Understanding

Schizophrenia is a debilitating, life-altering mental disorder that has been studied for over a century, and yet the cause of this condition is still unknown. The implication of understanding the initial trigger for a disease that affects over twenty million people worldwide could change countless lives.

My opinion: *Toxoplasma* infection is responsible for a substantial percentage of schizophrenics, but not all. How could anyone ignore such an enormous collection of physical evidence supporting the connection? Although I would like to believe schizophrenia is a solve-all, cure-all type of disorder, it appears to be much more complicated. However, *Toxoplasma* gives insight into this mental disorder. Continued research constructed from the foundation created by past discoveries may lead to the exposure of another less-known pathogenic parasite that also causes psychosis later in life. The possibilities are endless, but researchers have to first focus on building from the data we already have.

Prevention and Treatment

The opportunity to prevent disease in millions of people is something scientists and health care workers across the globe fight for

on a daily basis. Not only would this improve numerous lives, but from a political standpoint, it would save billions of dollars for families and taxpayers.

If toxoplasmosis results in the acquisition of schizophrenia, then foundations and laboratories concentrated on either disease could combine to focus on the same goal. Funds and ideas would be shared, united to discover the cure that kills two birds with one stone. Researchers could concentrate less on attempting to resolve the chemical imbalance of the brain and spend more time discovering a vaccine against *Toxoplasma*. How wonderful would it be to create an intramuscular vaccination to immunize all children at birth or all pregnant mothers against this parasitic invader? The conception of an efficient inoculation that lasted, for example, as long as a tetanus shot where people required a booster every ten years to retain immunity, would prevent, not one, but two worldwide diseases. With consistent dedication, there is even the possibility of both schizophrenia and toxoplasmosis being completely eradicated from the human population. What a wonderful triumph all communities, united, could relish in.

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