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# Parasitic Protozoa and Helminths: Biological and Immunological Challenges

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**Parasitic protozoans and helminths pose considerable medical as well as scientific challenges. Investigations of the complex and very different life cycles of these organisms, their adaptation to the obligate parasitic mode of life, and their ability to face the hostile host environment have resulted in many exciting discoveries. Invasion of host erythrocytes by plasmodial sporozoites and intact skin by schistosomal cercariae are outlined as examples of the elaborate mechanisms of parasitism. Isolation and characterization of single protective antigens or subunit vaccines from these two organisms are examined as models for vaccine development. Finally, developments in exploring gene regulation in protozoans and free and parasitic nematodes are briefly outlined.**

PARASITIC PROTOZOANS AND HELMINTHS REPRESENT TWO major groups of infectious agents that are responsible for considerable morbidity and mortality in human and animal populations. These agents have a wide geographic distribution and, as multiple infections are common in endemic areas, the total number of infections in humans far exceeds the world population. The distribution of some of these infections is expanding because of the increased prevalence of immunosuppressive conditions such as malignancies, chemotherapeutic medications, and retroviral infections. Approximately 1 billion people are infected by ascaris; 600 million by malaria-causing plasmodia and 300 million each by schistosomes and filariae (*1*). While information on morbidity and mortality is less complete, it is estimated that malaria alone causes 1 million deaths among children yearly and that other infections such as schistosomiasis and filariasis cause chronic debilitating conditions that are associated with loss of productivity and reduced life-span. Parasitic protozoans such as *Pneumocystis carinii*, *Toxoplasma gondii*, and *Cryptosporidium* are emerging as major opportunistic infections in those infected with HIV or in those with other immunosuppressive conditions (*2*).

In spite of this enormous public health impact, a staggering contrast exists between our knowledge of protozoan and helminthic

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infections and our understanding of infections caused by bacteria and viruses. Details of the biology, biochemistry, and molecular biology of viruses and bacteria have been examined extensively while similar studies of protozoans and helminths have lagged. Although there is no specific scientific reason for this disparity, the complex structure of protozoans and helminths and the fact that they are endemic mainly in less developed countries may provide partial explanation. Whatever the reasons for this gap, the past two decades have witnessed the scientific rediscovery of parasitic protozoans and helminths.

The ability of protozoan and helminthic organisms to establish themselves as parasites in multiple hosts and to overcome host defense mechanisms has become a central scientific challenge (*3*). These organisms are characterized by complex life cycles in which changes between free living and parasitic stages occur and each stage represents a different biochemical and antigenic structure. The discovery of antigenic variation in trypanosomes has resulted in a considerable shift in interest in parasitic protozoans (*4*). In fact, investigations of the underlying mechanisms of antigenic variation and the molecular basis for antigen switching in trypanosomes are bridging the gap between the status of studies on protozoans and the expanding horizon of molecular biology and are adding to our understanding of gene structure and organization in eukaryotes in general.

The scientific community has also focused on the expanding medical challenge posed by these organisms. For example, multiple drug resistance is emerging as the single most serious problem in malaria chemotherapy (*5*). Continued analyses of parasite proteins are needed to develop species-specific diagnostic tests, which are lacking for many parasite species (such as *Leishmania*) that differ in their pathogenicity. Furthermore, we still have no effective and safe chemotherapeutic agents against certain parasitic infections such as *Trypanosoma cruzi*, and *Cryptosporidium*.

This article will highlight some of the most important and exciting issues in research on parasitic protozoans and helminths. The goal is to use the major biological differences between these two groups of organisms to contrast mechanisms of adaptation to parasitism. We will use information gained from representative examples of parasitic protozoans (*Plasmodium* and *Trypanosoma*) and of a parasitic helminth (*Schistosoma*). Because of the relative ease of culture of protozoans in vitro, there has been considerable progress in our knowledge of their biology and molecular organization. In contrast, maintaining the life cycle of worms in the laboratory necessitates passaging the organisms in experimental animals. To obtain significant numbers of schistosomes a colony of the snail intermediate host has to be maintained and the parasite must also go through a phase in mammalian hosts such as mice or hamsters. No in vitro culture systems have as yet been developed to maintain and expand the population of a parasitic worm in vitro. Despite these difficulties, there also have been recent advances in the characterization of gene expression in parasitic helminths.

## Biology of Parasitic Protozoans and Helminths

Parasitism involves a specialized and dependent mode of life; as such, all infectious organisms are parasites (*3*). The life cycles of protozoans and helminths often involve differentiation through several morphologically and antigenically distinct stages (*6*). Protozoans and helminths that parasitize humans gain access to their bodies via multiple routes including ingestion, direct penetration of intact skin, or the bites of insect vectors. They inhabit specific locations within their human host. Because of their small size (4 to 400 μm), protozoans may reside intracellularly (*Leishmania*, *Toxoplasma*, and *Plasmodium*), intravascularly (*Trypanosoma*), or in tissues such as brain (*Toxoplasma*); or they may be located in the lumen of the gut (*Entamoeba*). The larger size of the multicellular parasitic helminths (several millimeters to several meters) partially dictates their final habitat in the human host. For example, the small schistosomes (1 to 2 cm in length) are found in the portal venous or vesical circulation whereas the large tape worms (several meters in length) parasitize the lumen of the gut.

Humans usually serve as the definitive host for many parasitic protozoans and helminths; however, the concept of biological vectors that also are intermediate hosts adds a special feature to this group of infectious agents (*7*). Passage through intermediate hosts may simply be a mode for transmission but often it is significant for the life cycle of the parasite. For example, sexual multiplication of plasmodia during malarial infections occurs only in the mosquito vector whereas asexual reproduction, essential for expansion of the parasite population, occurs in infected humans (*8*). The situation is reversed in schistosomiasis; sexual reproduction of the worms occurs in infected humans whereas asexual multiplication takes place in the intermediate host, the snail (*9*).

Although there are many fundamental biologic differences between unicellular and multicellular organisms, a broad distinction may be made between microparasites and macroparasites (*10*). Microparasites (including bacteria, viruses, and protozoans) exhibit high rates of direct reproduction in the host; however, macroparasites (helminths) generally have no direct reproductive capabilities within their definitive host (*11*). It follows therefore that protozoans increase their population inside their human host without the necessity of passage through the environment or another intermediate host. For example, many malaria sporozoites may be introduced in a human host during the bite of one infected mosquito. After differentiation and multiplication in liver cells (7 to 14 days), several thousand merozoites emerge to begin the erythrocyte phase, in which the parasites invade and mature within host red cells. Cycles of multiplication and reinvasion of host erythrocytes continues at regular intervals (1 to 3 days) and may lead to accumulation of enough parasites to result in significant morbidity (*12*).

In contrast, parasitic helminths (in general) do not multiply within the human host. Schistosome infection in humans is initiated via penetration of intact skin by the infective stage, cercariae, which are found in contaminated bodies of freshwater. The likelihood that a cercaria will mature into a worm of either sex varies with the host species but in susceptible hosts is never greater than 40 to 60%. The exact mechanisms underlying the inability of all cercariae to mature into adult worms are not known, but the phenomenon may relate to innate immunity exhibited by each host species (*13*). For a human host to acquire a pair of male and female worms, exposure to more than two cercariae is, therefore, necessary. Even if a pair of opposite sex were present, the mature adult worms residing in humans are incapable of increasing their population by any multiplication mechanism within the human host; they can only produce eggs, which do not mature into adult worms in humans. To increase the number of worms in a human host, exposure to additional cercariae is necessary (*14*). The schistosomes increase their population in the environment by multiplication in the snail intermediate host. This occurs when mature adult worms in humans mate and produce eggs that have to pass with excreta to the outside environment. If these eggs reach freshwater they hatch, releasing motile organisms that invade the tissues of snails where they multiply and differentiate into the infective cercariae.

This fundamental difference between protozoan and helminthic organisms also has certain implications in describing and understanding factors that are involved in regulating host-parasite rela-

tionship. For example, mathematical models for the patterns of transmission of these organisms must take into account these differences in life cycle (*15*). Prevalence models largely fit microparasites; they describe the fractions of the human population that are either susceptible, infectious, or immune. Helminthic infections, in contrast, are better fit by density models because of the aggregated nature of the infection in human populations (certain individuals carry a high load of parasites) and of the unreliability of prevalence as an index of intensity of infection (the number of parasites per infected person). Thus, the distribution of helminthic infections in any given community does not follow a statistically normal pattern. Most infected individuals harbor a low worm burden and only a small proportion carry the brunt of heavy worm counts; this pattern corresponds to a negative binomial relationship.

The difference in life cycle is also reflected in the different impact that micro- and macroparasites have on the host immune responses (*12, 16*). Both protozoans and helminths pose a significant challenge to the host because of the multiplicity of antigens they present to the immune system, but the rate of growth and multiplication of parasitic protozoans is considerably faster than in helminthic infections. While the consequences of this difference are not fully understood, it is reflected in differences in the ability of the host to mount an effective and protective immune response. For example, primary infection with *Leishmania tropica* is followed by immunity to reinfection (*17*), and injection of irradiated malaria sporozoites into experimental animals results in acquisition of resistance against subsequent challenge (*12*). In contrast, acquired resistance to helminthic infection, if it ever exists in nature, is a slow process that takes several years to be established. Evidence for acquired immunity against schistosomes and most other helminthic infections is based on observations of changes in age-specific intensity of infection in humans (Fig. 1). In most endemic communities that have been studied, intensity of schistosome infection increases with age, reaching a peak in individuals 15 to 20 years old. In older people, a dramatic reduction of intensity of infection is seen. This decrease in adults has been considered strong evidence for acquired immunity in humans, although it takes a decade or so to be clinically significant (and other explanations, such as changes in the pattern of exposure to infected water, are possible) (*18*). Additional observations in experimental animals and in infected individuals after chemotherapy strongly argue for a role of acquired immunity in regulating intensity of infection in helminthiases (*19*).

## Invasion and Adaptation to Parasitism

Invasion of cells or internal organs of the host, is one of the most significant biological characteristics of parasites in general. For protozoan and helminthic infections, this process takes on special importance because of their size and the complexity of their life cycle. In protozoan infections, invasion of only a very restricted set of cell types may occur, as in the case of *Plasmodium* and *Leishmania* parasites; other organisms (such as *Toxoplasma*) are less discriminating, invading various host cells including muscle, brain, and mononuclear phagocytes.

Malaria provides an example of a stage-dependent specificity of invasion (*12*). The sporozoites are the first stage of the parasite in humans; they only invade liver cells. The next stage, merozoites, invade only host erythrocytes; the specificity of this process is dependent on receptor molecules on the erythrocyte surface (*20*). A suggestion that merozoites of *P. vivax* require the presence of the Duffy blood group substance on the erythrocyte surface was obtained from studies performed many years ago. Some black individuals who were inoculated with *P. vivax* for therapeutic purposes failed to acquire infection while Caucasians receiving similar inocula did. These results were correlated to the absence or presence, respectively, of Duffy blood group substance on their erythrocytes. Evidence was later obtained from in vitro studies that Duffy-negative erythrocytes are resistant to invasion by *P. vivax* (*21*). This resistance was found to be species-dependent since Duffy-negative erythrocytes are susceptible to invasion by *P. falciparum* (*20*). The ability of *P. falciparum* to invade is related to the presence of glycophorin A on the erythrocyte membrane (*22*). Infection of red cells lacking glycophorin A or glycophorin A and B is considerably reduced. Nevertheless, the fact that several strains of *P. falciparum* can still invade such cells suggests that the parasite may use other membrane receptors (*23, 24*). Furthermore, invasion has been shown to be dependent on sialic acid residues on the erythrocyte surface receptors; erythrocytes were made less sensitive to invasion by prior treatment with neuraminidase.

Invasion by the merozoite proceeds through the formation of a junction between the anterior end of its surface and the cell membrane (*25*). The junction moves around the parasite, creating a vacuole through which the merozoite invades the host erythrocyte (*26*). Subsequent to invasion, the membranes of infected cells are altered significantly, including the appearance of electron-dense knobs on the erythrocyte surface. The significance of this process is not clear, although knob formation has been implicated in the pathogenesis of cerebral malaria by inducing red cell sequestration (*27*). This effective, though biologically simple, intra-erythrocytic localization of the parasite provides excellent protection against multiple host defense mechanisms and allows the organisms to grow, multiply, and proceed to infect another cohort of red cells.

A different model for evasion of host protective responses, antigenic variation, has been described in trypanosomiasis (*28*). The phenomenon of antigenic variation in trypanosomes is the key adaptive mechanism for parasitism; it is, however, not restricted to protozoan organisms as it has been observed in *Borrelia*, *Neisseria*, and several viruses (*29*). During chronic trypanosomiasis in humans,

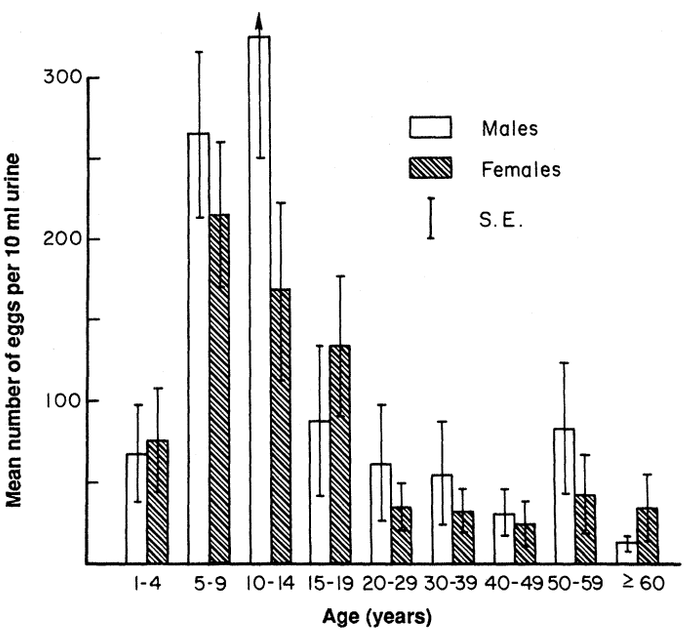


**Fig. 1.** An example of evidence used to indicate the existence of acquired immunity against helminthic infection. The histogram represents mean counts of *Schistosoma haematobium* eggs in 10-ml urine samples of the population of an endemic area (Kilole, Coast Province Kenya) (*71*). The sharp decrease in egg counts in individuals over 30 years may reflect acquisition of immunity.

waves of parasites appear in peripheral blood. This is particularly true in *Trypanosoma brucei gambiense* infection which causes the chronic Gambian form of sleeping sickness. These waves of peripheral blood parasites are serologically distinct; each represents a different antigenic variant and results in the formation of variant-specific antibodies. In trypanosomes, switching of surface glycoproteins is, however, not related to the presence of antibodies (*30*) suggesting that the process is due to programmed gene rearrangements that are independent of host or environmental factors. However, if the detected rate of antigenic switch in vitro ($10^{-5}$ to $10^{-7}$ per cell division) is extrapolated to the situation in vivo, rapid exhaustion of the parasite repertoire of variable surface glycoproteins would occur. Since this does not happen in vivo, other mechanisms besides antigenic variation may be involved in controlling the orderly appearance of cohorts of parasites in the bloodstream (*4*).

In spite of their size (approximately 200 to 600 μm), the infective stages of some worms are capable of penetrating intact human skin while transforming from free living to parasitic organisms (*31*). For schistosomes, the infective stage is the free living cercariae that are shed from the snail intermediate host into bodies of fresh water at ambient temperature. Each multicellular cercaria is bound by a syncytium that is limited by a traditional trilaminate plasma membrane. Upon encountering a mammalian host, cercariae attach to the skin and evacuate the contents of their pre- and post-acetabular penetration glands, which facilitates entry into the dermal and subdermal tissues (*32*). The process of invasion by the cercariae sets in motion a series of changes in the organisms that are aimed at adaptation to the environment of mammalian hosts. The cercariae are transformed into schistosomula, the first parasitic stage in humans or other mammalian hosts, which can live in physiologic salt concentrations at 37°C. The most dramatic morphologic change occurs in the surface membrane of the schistosomulum; the cercarial trilaminate membrane is converted into a heptalaminate complex structure composed of two closely apposed lipid bilayers (*33*). The surface of the schistosomulum (and all subsequent stages of maturing larvae and adults in mammalian hosts) is completely enclosed by the heptalaminate membrane, which shields the internal cellular structures of the parasite. The syncytium is connected by microtubule-lined intracytoplasmic processes to subtegumental cells. These cells contain multilaminate bodies that are thought to form the precursors of the heptalaminate surface membrane (*34*). The change from free-living cercariae to the parasitic schistosomula is usually completed within 3 hours and can be observed in vitro. The biological relevance of the membrane transformation has recently been demonstrated in studies with inhibitors of microtubule organization (*35*). Colchicine or vinblastine treatment of transforming organisms resulted in accumulation of significant numbers of multilaminate bodies in the subtegumental cells (Fig. 2). The surface of such treated schistosomula was limited by a trilaminate membrane (Fig. 3). In vivo survival of colchicine-treated organisms was significantly reduced, demonstrating that heptalaminate membrane formation is a prerequisite adaptation to the parasitic mode of life of schistosomula.

## Strategies for Vaccine Development

Induction of immunity is a key strategy for control of infectious diseases in general. Nevertheless, the task of producing vaccines against any of the clinically significant parasitic protozoan and helminthic infections has proven to be a tremendous challenge. One reason, which relates to their biologic and epidemiologic characteristics (*36*), is that a vaccine against a microparasite such as *Plasmodium* has to be almost 100% effective in the target population, in this case children less than 1 year of age. Furthermore, a significant proportion of this target population has to be vaccinated and protected if such a strategy is to succeed in either eradication of infection or reduction of its incidence to very low levels. In contrast, characteristics of parasitic helminths may allow the implementation of vaccination strategies in which vaccines are less than 100% protective. The observations that most helminthic parasites do not multiply within their definitive host, that a small proportion of infected individuals carry a high number of parasites, and that morbidity is related to the number of parasites suggest that a vaccine

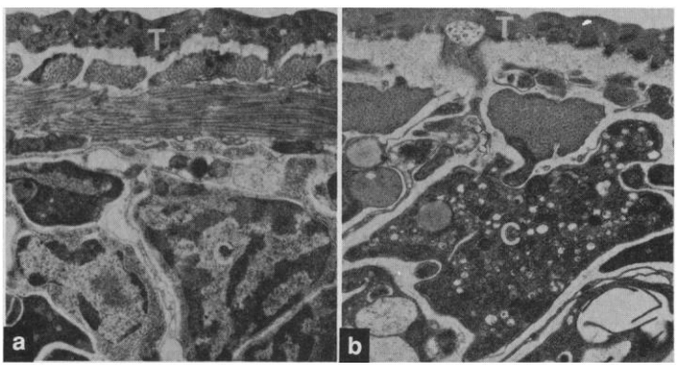


**Fig. 2.** Effect of colchicine treatment on the transformation of cercariae of *S. mansoni* into schistosomula. Transmission electron micrographs of (**a**) untreated schistosomula (final magnification, ×5100) and (**b**) schistosomula incubated during transformation and for 3 hours thereafter in colchicine (final magnification, ×5700). While very few multilaminate bodies were observed in the subtegumental cells of control organisms, large accumulations of these bodies were demonstrated in the perinuclear cytoplasm of cells in colchicine-treated schistosomula (C). These observations suggest that during transformation multilaminate bodies are guided or transported to the surface via microtubule-lined intracytoplasmic processes.

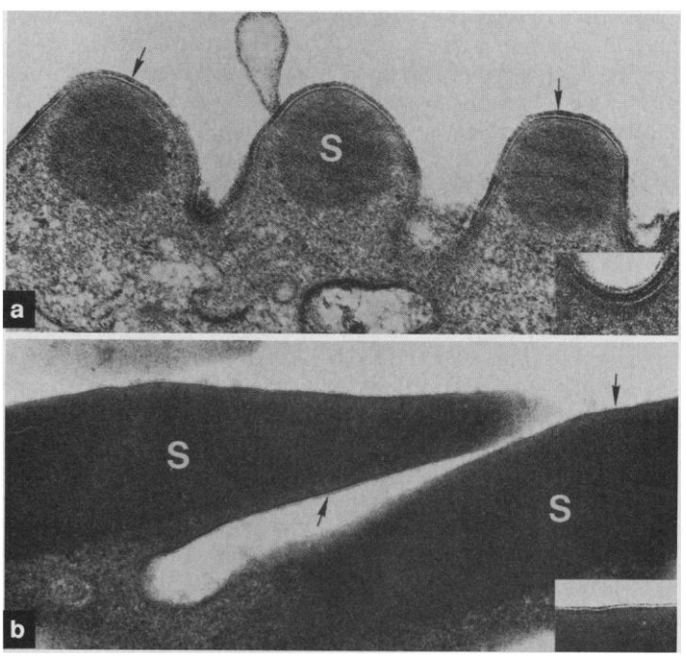


**Fig. 3.** Effect of colchicine treatment on the structure of surface membrane of cercariae transformation into schistosomula. Final magnifications: (**a** and **b**) ×69,000; insets, ×99,500. Panel a shows the surface of control schistosomula. "S" is a spine covered by heptalaminate membrane; the details of its structure is shown in inset. Panel b indicates spines "S" covered by a trilaminate membrane in a colchicine-treated sample; details are shown in the inset.

preparation less than 100% protective may be of major biological and clinical significance for controlling this group of infectious agents. Once the group of individuals with high intensity of infection can be identified, induction of partial protection in these individuals could dramatically decrease their contaminating effect on the environment and consequently reduce transmission to uninfected individuals. Furthermore, partial protection of heavily infected subjects would reduce their chances of becoming ill.

Until recently, induction of immunity against parasitic protozoan and helminthic infections has not been successfully achieved in humans or experimental animals except in limited studies with whole irradiated organisms or their crude extracts. Practically, there is only one example of consistently successful induction of protection in humans against a parasitic infection. For decades, it has been known that individuals who developed a scar from a primary infection with the protozoan *L. tropica* were immune to subsequent exposure to the same parasite. A more organized approach to induction of resistance to *L. tropica* by means of cultured organisms and measured doses is currently being used to protect humans (*37*). Other approaches to vaccination such as the use of irradiated infective stages of plasmodia, schistosomes, and hookworms have been successfully employed in inducing resistance in experimental animals and (in the case of malaria) in humans. It is usually necessary to attenuate, but not kill, the infective stages. For example, dead sporozoites are not internalized by cells and do not induce protective immunity (*38*).

The limitation of irradiation and similar techniques is related to the inavailability of sufficient materials. For example, it is a monumental task to develop an insectory for breeding enough mosquitoes to prepare irradiated malaria sporozoite vaccine for a small village in an endemic area. Furthermore, the multiplicity of antigens contained in these irradiated preparations adds another complicating factor since the corresponding host immune responses may not all be protective and may lead to untoward immunopathological or immunosuppressive consequences. Currently, a major approach to defining biologically relevant antigens involves production of monoclonal antibodies against stages of the parasite life cycle that may be involved in eliciting protective responses. In some instances (see below) these monoclonal antibodies have been shown to adoptively transfer partial protection to recipient animals and may, therefore, be helpful in characterizing the corresponding antigens.

Since malaria is the most clinically important parasitic protozoan disease in humans, the possibility of vaccine production has attracted considerable attention. For example, monoclonal antibodies against the circumsporozoite antigen of the malaria sporozoites (infective stage to humans) and against the erythrocytic parasites and gametocytes (infective stages to mosquitoes) have been produced (*39*). These antibodies were used to protect mice against subsequent challenge, prevent clinical disease, or block fertilization of the male and female gametocytes in the stomach of the insect vector. Extensive efforts have been directed at isolation, characterization, and testing of malaria antigens identified by these monoclonal antibodies. Furthermore, immunologically important epitopes of candidate malaria vaccines have been synthesized chemically or produced by recombinant techniques and have recently been evaluated in humans. However, to date no single preparation of a defined antigen or combinations thereof has resulted in uniform response in humans. Developing a vaccine for malaria is further complicated by the lack of a systematic knowledge of the nature of protective host responses, the nature of the effector mechanisms of resistance, and the possible heterogeneity of the causative organisms. Furthermore, most studies on vaccination protocols have used adjuvants, which adds another complicating dimension because of their known side effects and limitations of their use in humans.

The circumsporozoite (CS) protein of *P. falciparum* has received considerable attention since it was first recognized as being involved in immunity after animals are exposed to irradiated parasites (*40*). Sera from such immunized mice precipitate a 44-kD antigen (the CS protein) that reacts with protective monoclonal antibodies and constitutes the major protein on the parasite surface. The structure of CS protein and its encoding DNA sequence in several mammalian and human malaria parasite species have been identified (*41*). In *P. falciparum*, the CS protein contains 412 amino acids. The central region of the molecule is made of approximately 40 repeats of the sequence Asn-Ala-Asn-Pro (NANP). Repeats have been found in other malaria species but their detailed structure is species specific. The CS protein also has two highly conserved regions flanking the central repeats. Knowledge of the structure of the CS protein has facilitated a detailed examination of the host immune response to the different regions of the molecule and therefore the ability to construct subunit candidate vaccines. In humans and experimental animals, the immunodominant B cell epitope is the repeat region of the molecule (*42*). Antibodies to this region block sporozoite invasion of hepatocytes in vitro and adoptively transfer resistance in mice.

The humoral immune response to the repeat region may not be a sufficient protective mechanism for construction of a vaccine. Other cellular elements (such as cytotoxic T cells) and mediators (such as interferon $\gamma$) may be involved (*43, 44*). Only partial resistance can be achieved in animals adoptively transferred with antibodies to the repeat region and active immunization of humans with subunit repeat vaccine was only successful in two of nine individuals (*43*). Evidence also has recently accumulated that indicate a role for T cell responsiveness in vaccine-induced immunity to malaria. Immunity induced by malaria sporozoites is adoptively transferred by T cells from immunized to normal recipients. In addition, sporozoite immunization does not protect athymic nude mice (*44*). Recently, systematic examinations of T cell responses in immunized animals and in infected humans have been conducted. In one study (*45*), mice that had been immunized repeatedly with irradiated sporozoites were injected with antibodies to either CD8 or CD4 determinants (to deplete the corresponding T cells) and were then exposed to live sporozoites. The absence of $CD8^+$ but not $CD4^+$ cells resulted in abrogation of acquired immunity (*45*).

Examination of the T cell response and its genetic restriction in immunized mice has resulted in identification of the Th2R region (amino acids 326 to 345) as a helper T cell site. Similarly, human T cell epitopes of the CS proteins have been examined in studies with overlapping synthetic peptides corresponding to different areas of the molecule. In one such study, the ability of 29 synthetic peptides (covering the entire CS protein) to stimulate peripheral blood mononuclear cells of individuals from a *P. falciparum* endemic area was examined (*46*). In a significant number of individuals, recognition of residues corresponding to the Th2R region of the molecule occurred. In another study, approximately 70% of samples tested reacted with synthetic peptides corresponding to Th2R and Th3R regions of three strains of *P. falciparum* (*47*). In addition, human T cell reactivity to Th3R correlates with resistance to *P. falciparum* (*48*). An outcome of these mapping studies is the recognition that T cell epitopes of the CS protein are located in the polymorphic segments of the molecule. Examination of intra-species variation of the CS gene in several *P. falciparum* isolates demonstrated substitutions in biologically important regions such as Th2R, suggesting that strain variability in CS protein is significant (*49*). These findings indicate potential serious complications for vaccine production against malaria.

The task of developing defined vaccines for helminthic infections may be far more challenging. However, convincing evidence for the

existence of acquired resistance against schistosomiasis, filariasis, and other helminthic infections in humans has been obtained (*50*). The development of specific anti-eosinophil sera and in vitro systems to examine antibody-dependent cell-mediated cytotoxicity against *Schistosoma mansoni* schistosomula and larval stages of other helminths demonstrated the pivotal role of eosinophils as well as antibodies and complement in host resistance (*51*). Other effector mechanisms, particularly those resulting in activation of the mononuclear phagocytes, are also involved (*52*). The increased understanding of effector mechanisms has led to several approaches to induction of resistance against multicellular parasites in experimental models. Irradiated organisms, protective monoclonal antibodies, single purified or recombinant antigens, and anti-idiotypic and anti–anti-idiotypic antibodies have all been shown to induce significant in vivo resistance against schistosomiasis (*53*). It has to be noted however, that protection achieved has only been partial. Even with the use of several adjuvants, no single antigen preparation has resulted as yet in complete protection against any helminth infection. The basis for this limitation is unknown.

Isolation and characterization of defined helminthic antigens with potential protective effects has been dependent either on examinations of crude parasite extracts or the development of monoclonal antibodies capable of adoptively transferring resistance to normal recipients (*54*). In studies with crude extracts of *S. mansoni* schistosomula, significant protection against subsequent challenge was observed (*55*). The antibody response in animals vaccinated with extracts has been used to identify individual active antigen in the crude preparations. Protective *S. mansoni* antigens of 97, 68, and 43 kD have been isolated (*55*).

The purified 68-kD schistosome glycoprotein induces significant protection by reducing worm load by 22 to 66% upon challenge of immunized mice; this level of resistance was induced without the use of adjuvants. Administration of the 68-kD antigen to mice did not result in sensitization to subsequent injection of schistosome eggs, which suggests that the use of such antigen in infected animals or humans will not lead to exacerbation of egg-related immunopathology. Immunolocalization studies have revealed that the 68-kD antigen is associated with cytoplasmic granules in the head and preacetabular glands of cercariae and schistosomula (*55*). The DNA sequences encoding this antigen and thus, its amino acid sequence are now being determined.

Other protective schistosome antigens have been isolated; some have been cloned and their immunologic epitopes have been mapped (*56*). Synthetic peptides corresponding to highly hydrophilic or mobile areas of the 28-kD antigen have been used to examine the B and T cell epitopes (*57*). Two peptides were identified as major immunoglobulin (IgG) targets including one (corresponding to amino acids 24 to 43) that is the target for the IgG2a antibody that mediates eosinophil-dependent cytotoxicity against schistosomula in vitro. As in the case of malaria subunit vaccines, these studies pave the way to examine in detail the most optimal structure of candidate anti-helminthic vaccines. Furthermore, approaches such as purification of schistosome paramyosin either immunologically or biochemically or the parasite glutathione transferase have demonstrated the potential protective effect of these molecules (*58*). In other studies, the possibility of utilization of anti-idiotypic as well as anti–anti-idiotypic antibodies to induce significant protection has been reported (*59*).

Similarly, in lymphatic filariasis, it has been demonstrated that a crude extract of microfilariae of *Brugia malayi* induces resistance to subsequent injection of the parasite larvae in mice or to exposure of rodents of the genus *Meriones* (called jirds) to the infective stage (*60*). The limited nature of antibody response in immunized animals led to partial purification of several putative protective antigens. One of these is an ~60 kD antigen that has recently been examined in detail; a recombinant cDNA clone has been isolated (*61*). Insertion of this clone in vaccinia has been achieved and the recombinant virus induces significant resistance in jirds.

Induction of resistance to a multicellular organism such as the schistosome by a combination of several pathways is essential because of the complex nature of the parasite and because of the partial protection induced by each pathway. We now have to explore the possibilities of multiple antigens and custom-made adjuvants as complementary strategies for the development of effective vaccines.

## Gene Regulation

While this review has focused on some features that characterize adaptation to parasitism in protozoan and helminthic infections and new insights gained in vaccine production, it should be noted that considerable progress has been made in understanding the molecular aspects of gene regulation in these organisms. Parasitic protozo-

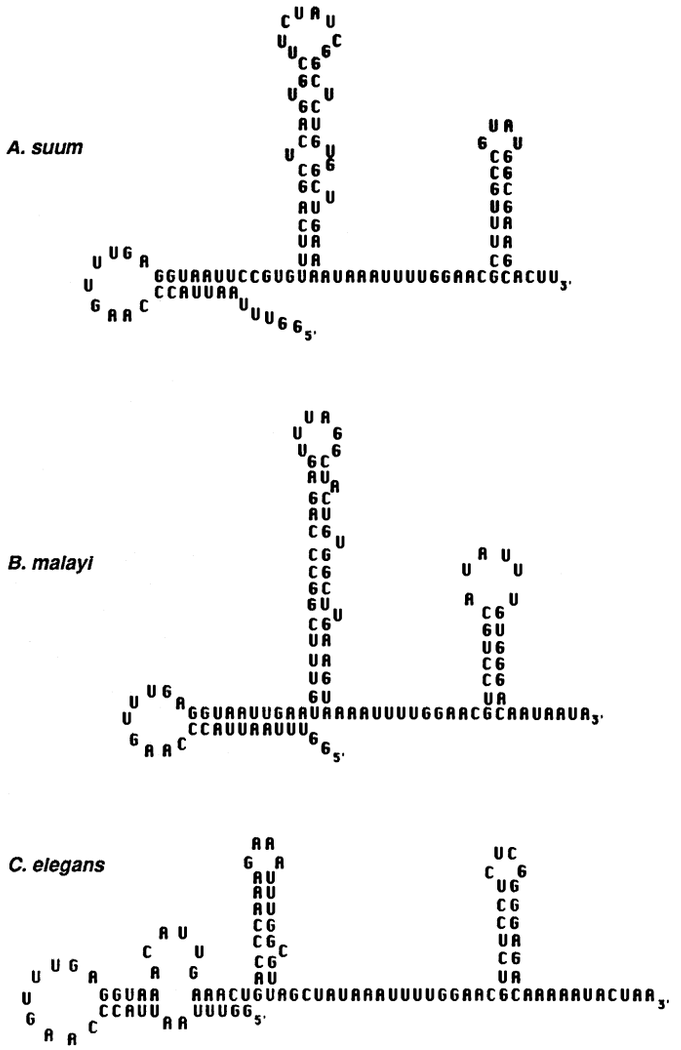


**Fig. 4.** Potential secondary structure of spliced leader RNAs from the free-living nematode *Caenorhabditis elegans* as compared to those of two parasitic nematodes *Brugia malayi* and *Ascaria suum* (*69*). The computer program of Zuker and Stiegler (*72*) was used to generate the possible secondary structures. Comparison with structures of spliced leader RNAs of other parasitic protozoans has been described (*70*). Each contains three stem loops and a 5′ splice site is next to the turn of the most 5′ loop. An Sm-binding sequence is located between stem loops 2 and 3.

ans and helminths provide excellent models for studying cellular differentiation. Because of the obligate parasitic nature of these organisms, they are programmed to switch different stages of their life cycle between free-living forms in the environment and parasitic forms in one or more hosts. In many examples switching means a change in morphologic, biochemical, and antigenic structure of the organisms. Switching has to be regulated at the molecular level, often via elaborate mechanisms. Studies of gene regulation in protozoan and helminthic organisms have demonstrated some intriguing similarities and many differences and have uncovered an unexpected wealth of information relevant to eukaryotic gene regulation.

Switching of antigenic variants in trypanosomes involves control of the expression of the antigenically distinct variable surface glycoproteins (VSGs), such that only one variant is transcribed at a given time. There are approximately 1000 VSG genes per trypanosome genome (*62*). Switching is accomplished primarily through gene conversion, in which an unexpressed VSG gene is transferred to a telomeric "expression site" located on another chromosome (*63*).

Another facet of gene expression in trypanosomes that is the focus of considerable attention is trans-splicing (*64*). Messenger RNAs in trypanosomes consist of two exons that are transcribed from separate genes and are spliced post-transcriptionally. At the 5′ end of each mature mRNA is a capped, 39-nucleotide (nt) sequence called the mini-exon or spliced leader (*65*). The mini-exon becomes joined to a main exon that was cut of a polycistronic unit. The conclusion that mRNA maturation in trypanosomes occurs by trans-splicing rather than by the conventional cis-splicing mechanisms was based on the observation that a free 100-nt "intron" (or "transon") is released from poly A–fractionated trypanosome RNA after treatment with a debranching extract of HeLa cells (*66*). This 100-nt piece indicates that trypanosome transcription proceeds through a Y-branched intermediate, rather than through the lariat structure observed during cis-splicing.

Only recently, have comparable studies been possible in helminthic organisms. These organisms also use trans-splicing in the maturation of some mRNAs. A 22-nt spliced leader was reported on some, but not all actin mRNA in the free-living nematode *C. elegans* (*67, 68*). The leader RNA is not polyadenylated and is only found on a subset of messages; this is in contrast to the trypanosomes, where the spliced leader has been found on all messages.

An identical 22-nt spliced leader has been described in two medically important namatodes *B. malayi* and *Ascaris* (*69*). The orientation of the 22-nt piece within the 5*S* genes of *B. malayi* and *Ascaris* is opposite to that seen in *C. elegans*; the effect of this difference on function is unknown. Although there is little primary sequence identity between the spliced RNAs in different nematodes or in other organisms such as trypanosomes, they can assume identical secondary structures; each is folded into three stem-loops (Fig. 4). Comparison of these loops with previously reported secondary structure of spliced leader RNAs of other organisms (*70*) suggest similarities with mRNA assembly and biogenesis in vertebrate cells.

In conclusion, this review concerned selected aspects of the biology, immunology, and molecular organization of parasitic protozoans and helminths. These organisms pose a significant challenge to the scientific community. Future research efforts need to address two major fundamental areas: examination of the molecular basis for adaptation to parasitism and development of newer control measures including drugs and vaccines.

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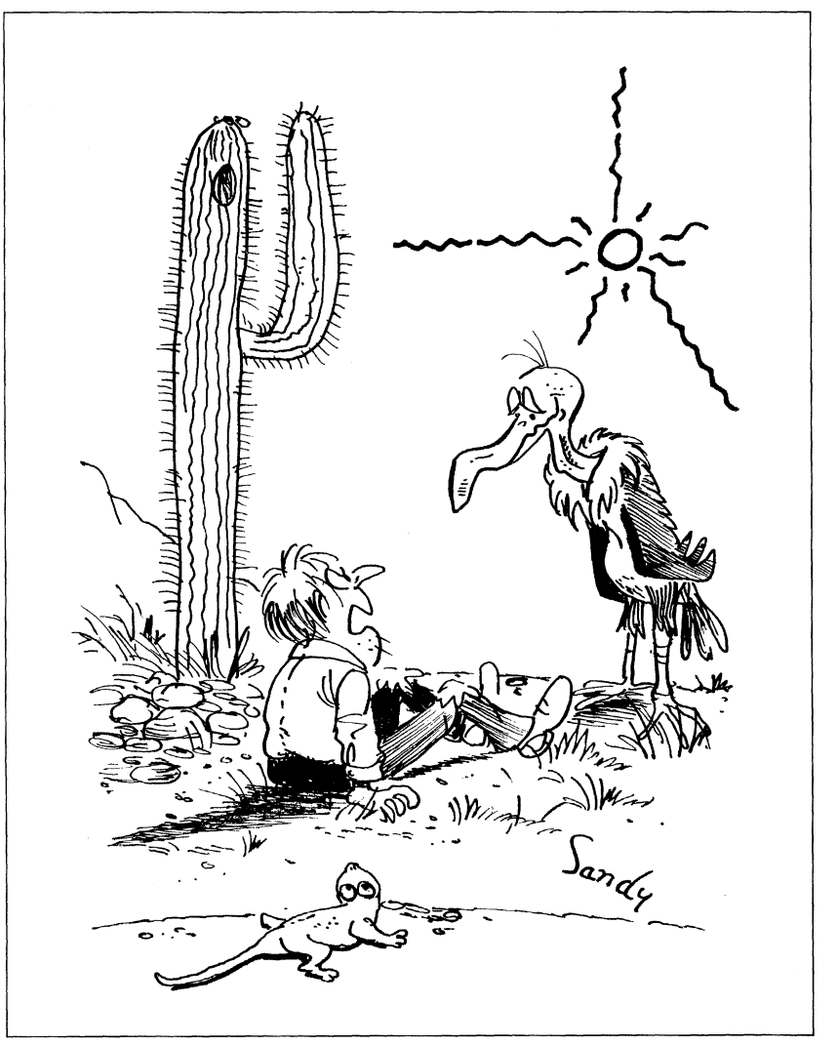


***"That's a lie! You are <u>not</u> the blue bird of happiness!"***