CELL INJURY AND PARASITIC INFECTION

SOMPHONG SAHAPHONG

Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

INTRODUCTION

To start with, the concept of cell injury will be briefly discussed. The dominant concept which regards disease processes as the results of the reactions of cell to injury was first introduced by Professor Rudolf Virchow in 1858. This was only a few years after the cell concept was introduced by Schleiden and Schwann. The introduction of the cell concept into the study of disease has proved to be one of the most important developments in modern medicine. Reviews of various aspects of cell injury have been published by several investigators (Cameron, 1951; De Reuck and Knight, 1964; Farber and Magee, 1965; Trump and Ericsson, 1965; Trump and Ginn, 1969; Trump *et al.*, 1969; Trump and Arstila, 1971; Trump *et al.*, 1971; 1973).

Concept of cell injury

Trump and Ginn in 1969 defined an injury as any physical or chemical stimulus that perturbs cellular homeostasis. It can also be thought of as a process which change the entropy level of the steady state. The diagram in Fig. 1 is very useful in visualizing the effects of injury on cell. In this simple diagram, the time is plotted along the abscissa and the level of homeostasis is plotted along the ordinate. The cell is depicted in a normal steady state at the normal level of homeostatic ability. Some injuries are sublethal even if prolonged, and the cell is able to adjust to the presence of continued abnormal stimuli by reaching some altered steady states. Thus, homeostasis can be maintained at different level but enough to

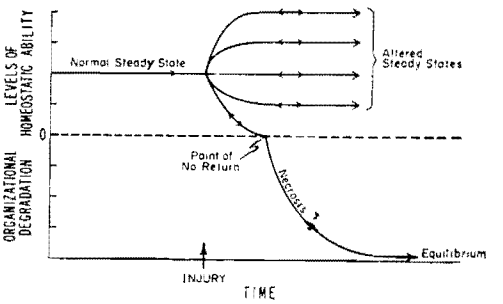


Fig. 1—Diagram showing a conceptualization of the effects of injury on a cell; both lethal and sublethal injuries are shown (From B.F. Trump and F.L. Ginn, 1969).

sustain life. Examples are hyperplasia, fatty metamorphosis, hypertrophy and atrophy. This adaptation of a cell is of great importance in understanding human diseases. An acute lethal injury is the injury which is followed by the death of the cell. Following a lethal injury to the cell, two phases can immediately by arrived at on the basis of logic. These two phases are the phase prior to the timecell has lost the ability to recover if the injurious stimulus is removed and the phase when it cannot recover even if the stimulus is removed. The point separating the two is often called the point of no return of the point of cell death. This term was first used by Majno in 1964. The changes prior to this time are reversible. The irreversible phase is sometimes referred to as necrosis and involves a variety of processes, including mitochondrial degeneration with calcium accumulation autolytic breakdown due to lysosomal enzymes and other factors such as low pH. Cell death may be defined as irreversible loss of integrated cell activity resulting in an inability to maintain homeostatic me-

chanism. Necrosis refers to the subsequent degeneration of a dead cell into component molecules which gradually approach physico-equilibrium cell death, therefore is to be distinguished from necrosis. Cell death may or may not be followed by necrosis. For example cells that die of ischemia in the body undergo necrotic changes whereas cells killed by immersion in formaldehyde do not, because of the inactivation of enzymes and stabilization of structural components.

Effects of injury on cells

Fig. 2 shows a general conceptualization of the life of cells and the effects of injury on them. Two principal types of cells: dividing cells and nondividing cells are shown. Examples of dividing cells are primitive cells in the bone marrow, primitive germ cells and precursor cells in the intestinal tract. Nondividing cells are cells that are not in the mitotic cycle and are normally assumed to be in prolonged G_1 phase.

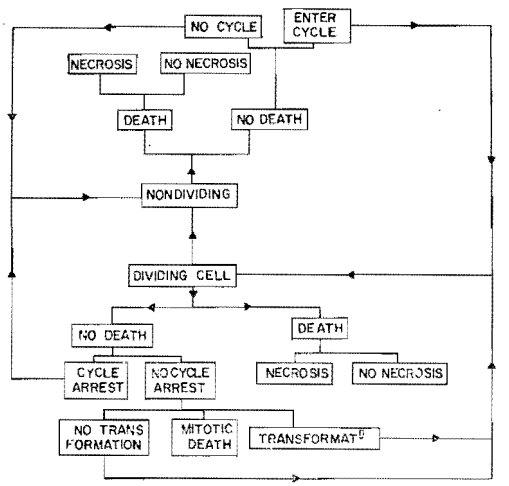


Fig. 2—Diagram showing the possible effects of injury on dividing and nondividing cells (From Trump and Mergner, 1974).

Following injury a nondividing cell either dies or does not die. If it dies, it may or may not undergo necrosis, depending on the type of injury. Nondividing cells that do not die may either reenter the cycle and become dividing cell or may not enter the cycle.

In the case of dividing cells, certain cells leave the mitotic cycle, during normal development, and become nondividing cells, whereupon they are subjected to the rules applying to nondividing cells. Like a nondividing cell, following injury a dividing cell either dies or does not die. If it does not die, the mitotic cycle may become arrested, whereupon the cells become subject to the rules of nondividing cells or cycle arrest does not occur. Several possibilities exist in the case of no cycle arrest. These are malignant transformation, mitotic death or continue division without transformation.

Stages of cell injury

Observations made in our laboratory as well as reviews of numerous publications dealing with ultrastructural aspects of several disease including parasitic infections reveal a variety of ultrastructural changes. In spite of a huge and bewildering array of ultrastructural changes, however, careful examination showed a limited number of patterns which are common to many types of injury and many types of disease processes. As a result, the hypothesis of a final common pathway of progressive pathophysiologic and ultrastructural changes from normal steady state through sublethal or reversible states to an irreversibly altered phase following injury has been proposed (Trump and Ginn, 1969; Croker *et al.*, 1970). The phase extends from initiation to the point of no return and characterized by a sequences of changes in the cell which is designated as stages 1, 2, 3, 4 and 5. Characteristics of theses changes have been previously defined (Trump and Mergner, 1974) and will be briefly summarized here.

Stage 1, is the normal condition of cell. The cellular junctional complexes and the cell membrane are intact, the latter shows a typical trilaminar appearance at high magnification. Endoplasmic reticulum and nuclear envelopes appear collapsed or slightly enlarged with ribosomes attached to the membranes. Mitochondria exhibit orthodox configuration with intact inner and outer membranes and a moderate dense matrical compartment containing granules. The cell sap shows moderate density and contains clusters of free ribosomes. Nuclear chromatin is regularly diffuse and lysosomes have intact membranes.

In stage 2, the nuclear chromatin is clumped and there is reduction in cell glycogen, the mitochondrial granules disappear. The endoplasmic reticulum is dilated. Ribosomes or polysomes may or may not be seen attached to the surface of the endoplasmic reticulum membrance. These changes correspond to the changes of so-called hydropic degeneration. The cell surface contours are distorted with small blebs developing along the plasma membrane and the cell sap may be swollen.

In stage 3, there are in addition to the above changes, prominent contraction of inner mitochondrial compartments. The mitochondrial matrices appear dense and the space between the inner and the outer of the envelope and the cristae is enlarged.

In stage 4, in addition to the above changes, some mitochondria show swelling of the inner compartment while other show condensation. In some cells such as renal proximal tubular cells some mitochondria show both types of changes.

In stage 5, in addition to the above changes, all mitochondria are swollen and the inner compartments are expanded often with interruption of the outer membranes. Tiny flocculent densities as well as annular particulate bodies are seen within the swollen mitochondria. The lysosomes begin to disappear. There are numerous interruptions in the continuity of the plasma membrane and of the nuclear envelope.

The rapidity of appearance and duration of each of these stages are highly variable. Stage 3 can be transient in some cell types and stage 4 may or may not always be seen in every cell type or at every temperature. Stages 2 and 3 are reversible stages of cell injury and seem to be compatible with continued survival. However, stage 5, though a completely necrotic cell cannot initially be differentiated by light microscopy using hematoxylin and eosin stained sections of tissue fixed in formaldehyde. A cell can be in this necrotic stage for several hours, perhaps eight or more, without showing any discernable chage by light microscopy. Studies on morphological changes in the cell of several parasitic infections both in our laboratory as well as from the work of other investigators, a sequence of changes at cellular and subcellular levels can be recognized. It is remarkable how similar these changes are with various types of parasites in various types of cell though the initial interaction may vary.

One of the most common finding in several parasitic infections is the stimulation of the process of phagocytosis. It is thought by several investigators (Trubowitz and Masek, 1968), that it is the most important factor in removing parasites from the blood. This process is known to link closely with the lysosomal system. Diagram in Fig. 3 is modified from that of Trump and Mergner (1974). It illustrates both the lysosomal system and the process of phagocytosis which further divides into heterophagocytosis and autophagocytosis. First let us consider the lysosomes. These are special group of cellular organelle having single membrane bound and contain several acid hydrolases inside. Enzymes or acid hydrolases are synthesized

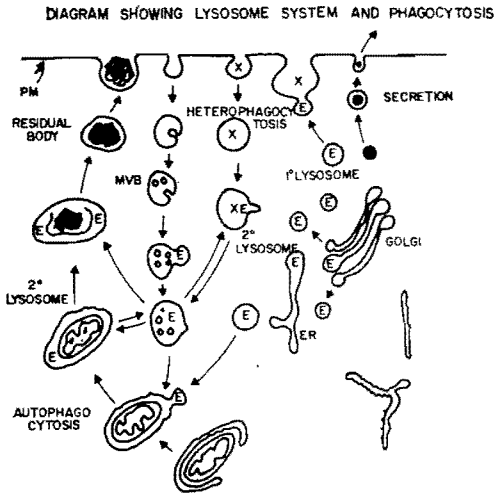


Fig. 3—This is a diagram showing lysosomal system in close relationship with phagocytosis. The enzyme is indicated by E, and the substrate by X. ER, endoplasmic reticulum; MVB, multivesicular body; PM, plasma membrane.

at rough endoplasmic reticulum and transported to the Golgi apparatus where they become concentrated. Subsequently, vesicles with single membrane bound containing acid hydrolases pinch off. These newly formed lysosomes are called primary lysosomes or virgin lysosomes. The term GERL was used by Novikoff (Novikoff *et al.*, 1964) to describe the organelles involved in the formation of the lysosomes.

Phagocytosis

Heterophagy is a mechanism by which macromolecules or macromolecular complexes are actively taken up by the cell and transported to the lysosomes (Cohn, 1971). Often the initial step of this mechanism is known as phagocytosis, pinocytosis or collectively termed as endocytosis. In the first part of this process, cell extends small cellular expansions or pseudopods which become closely applied to the surface of the attached particle or material. These processes meet and fuse around the particles, thus forming the so-called phagosome which is drawn into the cell. In the subsequent stages lysosome hydrolases are transported into this vacuoles from the Golgi apparatus by so-called primary lysosomes which fuse with the heterophagosome and release the hydrolases into the vacuoles (Novikoff *et al.*, 1964); deDuve and Wattiaux, 1966).

Autophagy is the phenomenon in which the lysosome system digests the cell's own materials (deDuve and Wattiaux, 1966; Ericsson, 1969). The phenomenon of autophagocytosis is similar to heterophagocytosis. In this instance the vacuole appears to form by exotropy into the cisternae of endoplasmic reticulum or other portion of the cytocavitary network (Ericsson and Trump, 1964; Arstila and Trump, 1968). This results in the enclosure of a portion of cytoplasm, such as mitochondrion in a double walled sac. Enzyme is added by fusion of this autophagosome with primary or secondary lysosomes. Both autophagy and heterophagy are apparently energy requiring processes which utilize ATP or other high energy intermediates, but the force involved in these membrane movements are not fully understood. For the fate of the materials inside the lysosomes, if the materials can be digested by lysosomal enzymes, little remains inside the lysosome, since the smaller hydrolyzed products will diffuse to the cell sap and used for other purposes. However, in chronic injury including some parasitic infections this is not always the case. The ingested material may be indigestible and may remain for a long time before it is completely digested. Also the uptake of foreign material may be so extensive that the reservoir for the acid hydrolases cannot digest all incoming materials, which will therefore accumulate inside the lysosomes. Furthermore, the cell production of acid hydrolases or their transportation into the phagosome may be injured, thus also leading to the accumulation of materials inside the lysosomal

system. In some cells, the debris-laden vacuole will move back to the plasma membrane and in the process reversing that by which the phagosomes are formed merge with it and eject its contents into extracellular space. It is not known whether all cells do this. Those of kidney tubules and liver certainly do ejecting the contents of their lysosomes into the lumens of the tubules and bile canaliculi respectively. Other cells such as neurons and cardiac muscle cells may lack this capability, it is believed that the aging pigment or lipofuscin that accumulates with time in these cells may derive from such debris.

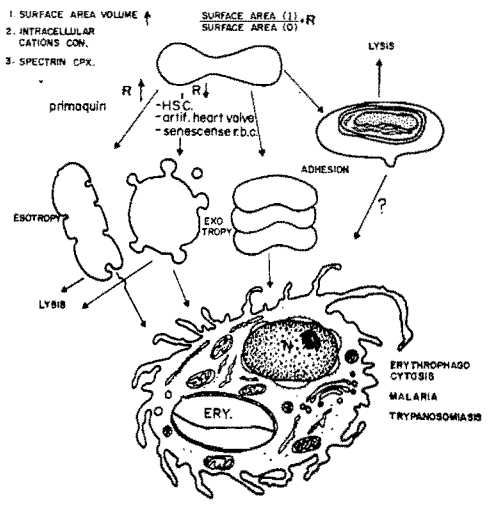


Fig. 4—Diagram showing various pathological changes of erythrocytes following malarial infection. Note also the erythrophagocytosis by macrophage at the bottom of the diagram.

Malarial infection as a model for cell injury

Figs. 4 & 5 depict normal erythrocytes and several pathologic erythrocytes observed in

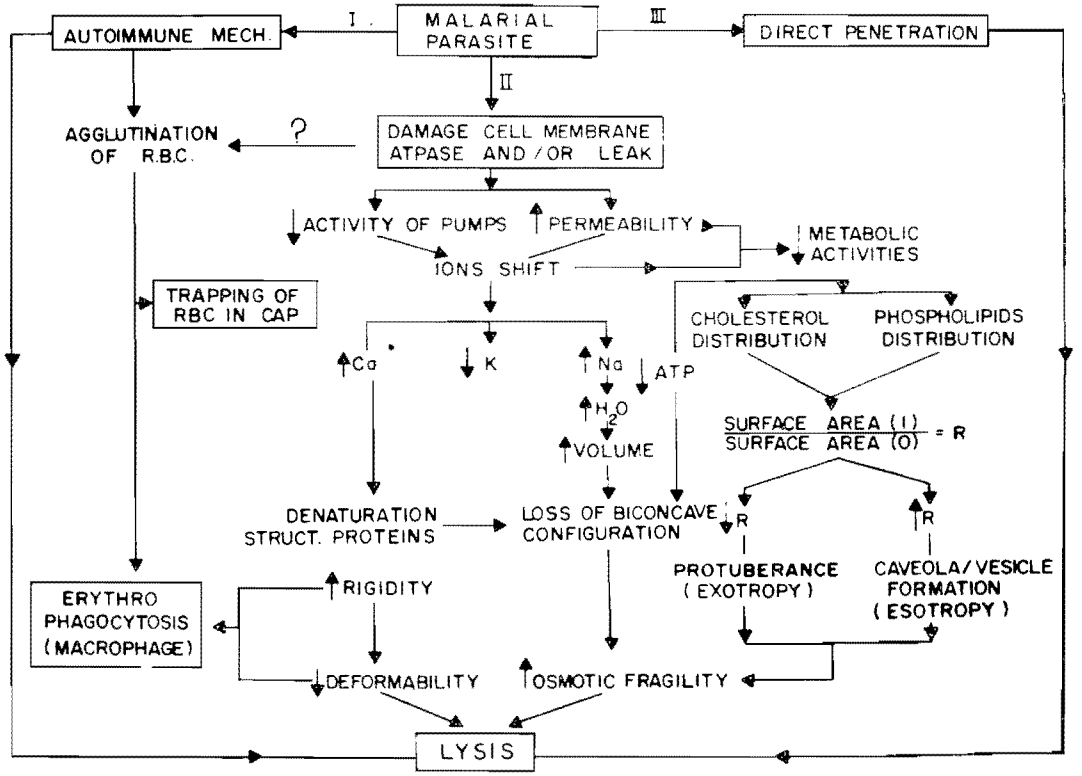


Fig. 5—Diagram illustrating 3 possible mechanisms causing lysis of erythrocytes by malarial parasites. These are autoimmune mechanism (I), the release of cytotoxic factor causing damage to erythrocyte membrane (II) and direct penetration of the parasite into the cell (III).

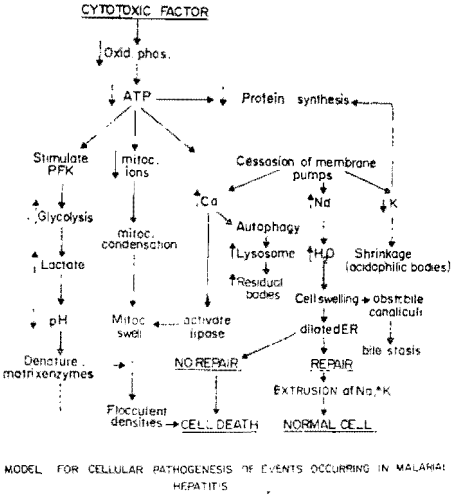
malarial infection. At a steady state, erythrocyte shape is biconcave. Several considerations are believed to involve in the control of this steady state: these are high ratio of surface area over volume (Tosteson, 1964), intracellular cations concentrations (Weed *et al.*, 1969), the presence of spectrin complex which is an acto-myosin like proteins and is responsible for controlling the red cell shape and motility by forming some kind of polymerization or network formation induced by adenosine triphosphate, the R value which is expressed as the ratio of the surface area of the inner leaflet of the membrane over the surface area of the outer leaflet of the membrane (Sheetz and Singer, 1977; Birchemeier and Singer, 1977). RBC change in malaria occurs primarily in the parasitized cells, later on in the course of infection changes also occur in the non-parasitized cells. Changes in the surface of the erythrocyte membrane induced by malarial infection into any of these 4 types enhance the process of erythrophagocytosis by the macrophages in the spleen and in the bone marrow (Conrad, 1966).

It is well known that individual species of malarial parasites are highly host specific (Garnham, 1966) but the mechanism determine susceptibility or innate resistant are poorly understood. Complete invasion process of erythrocyte by parasite requires 2 receptors (Miller *et al.*, 1975) one for the attachment of the parasite to the membrane and the other for interiorization of the parasite into the cell. The exact nature of these receptors is not clear at the present time. However, they are known to be sensitive to the treatment with trypsin, chymotrypsin pronase and in some case with neuraminidase (Miller *et al.*, 1973). Thus, it is highly suggestive that they are at least composed of proteins if not completely in part.

Erythrophagocytosis first described by Kolliker in 1849, is the mechanism by which aged and atypical erythrocytes are removed from the circulation by cell of the reticuloendothelial systems, particularly the macrophages in the spleen and the bone marrow. Erythrophagocytosis is also one of the process by which the erythrocytes are removed after haemorrhage. In both instances the erythrocytes are broken down by lysosomal enzymes. In the former case, most of the iron released forms ferritin and is reutilized, while in the latter case much of the iron is converted to hemosiderin and stored for indefinite periods of time in residual bodies called siderosomes. The cellular events in erythrophagocytosis are the same as previously described in phagocytosis.

In the late stages of malarial infection a humoral cytotoxic substance not fully yet categorized by with the molecular weight of less than 1000 has been demonstrated in the blood in *P. knowlesi* and *P. berghei* infections (Maegraith, 1968). This factor inhibits respiration and oxidative phosphorylation of mitochondria of the hepatocyte as well as the isolated liver mitochondria. Mitochondria in the cell of the liver in the late stages of infection showed structural damage. This factor is diffusible and arises from somewhere in the parasite erythrocyte complex. Maegraith (1968) found that it may act synergistically with anoxia and ultimately produce cell death.

Fig. 6 shows working hypothesis of the sequence of events in hepatocyte following malarial infection. Oxidative phosphorylation of mitochondria of the hepatocyte is inhibited by the so-called cytotoxic factor resulting in a drop of ATP level. The drop in cellular ATP stimulates the activity of phosphofructokinase, and this results in an increased rate of anaerobic glycolysis. Accelerated glycolysis leads to the accumulation of lactate, which together with the increased inorganic phosphate, reduces the intracellular pH. The change in intracellular pH is



MODEL FOR CELLULAR PATHOGENESIS OF EVENTS OCCURRING IN MALARIAL HEPATITIS

Fig. 6—Working hypothesis of the sequence of events in the hepatocyte following malarial infection and the release of cytotoxic factor. ATP, adenosine triphosphate; oxid. phos., oxidative phosphorylation; PFK, phosphofructokinase.

known to be associate with clumping of nuclear chromatin and denaturation of mitochondrial matrical enzymes. Other effects of reduced ATP concentration are interference with sodium, potassium and calcium pumps at the cell membrane and impairment of protein synthesis. Ion shifts occurs, sodium leaking into the cells, followed by calcium, potassium leaking out.

The increased sodium content soon leads to swelling of various cellular compartments such as cell sap and the endoplasmic reticulum. This is because of the water influx that accompanied the sodium and also total cation flux because of the Donnan effect. Bile statis is thus believed to be the result of the swelling of bile canaliculi causing obstruction. Clumping of nuclear chromatin, dilated endoplasmic reticulum and dilated cell sap as mentioned earlier are reversible changes that can be repaired following removal of the initiating event. Extrusion of sodium and increase potassium are followed by return to a normal cell. Increase in calcium in cell stimulates the process of autophagocytosis (Trump *et al.*, 1971) which follows by increase in lysosomal activity and increase in number of residual bodies. Mitochondrial release of calcium can also be mediated following cyclic AMP action from hormone such as glucagon (Shelburne *et al.*, 1973 a, b). Another consequence of calcium dispersion in cytosol is the activation of phospholipases (Boime *et al.*, 1968). These attack the mitochondrial inner membranes resulting in release of fatty acids and changes in membrane integrity. Fatty acids are known to be uncoupler and mitochondrial swelling agents. In early phase, the effects can be repaired, altered mitochondria showed only marked inner compartment swelling. In later phase, however, the changes are irreversible with development of flocculent densities within mitochondrial matrix and rupture of mitochondrial outer membrane.

The conceptualization of hepatitis in malarial infection, thus, includes the sequence of events initiated by cytotoxic substance-mitochondria interactions leading to involvement of many organelles. Ions and water movements within the cytoplasm play the key role in the pathogenesis of cell death as described above.

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HUMORAL IMMUNE RESPONSE IN PARASITIC INFECTIONS

STITAYA SIRISINHA

Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

The fact that clinical evidence for acquired immunity in parasitic infections is often absent or equivocal at best has prompted the belief that parasites, unlike bacteria and viruses, are poorly immunogenic, and are not effective in inducing the immune response in affected hosts. However, it has been shown that both humoral and cellular immune components are stimulated during the course of infection by most parasitic agents that have been thoroughly investigated (Ogilvie, 1970; Soulsby, 1972; Porter and Knight, 1974; Mauel and Behin, 1974; Cohen and Sadun, 1976). Both types of responses however correlate poorly with protection, suggesting that neither antibodies nor sensitized cells could damage the parasites. Such a conclusion is largely supported by the data showing that passive serum or cell transfer often fails to induce immunity in recipients (Ogilvie, 1970; Mauel and Behin, 1974; Porter and Knight, 1974; WHO, 1975). However, because both parasiticidal antibodies and sensitized cells can also be demonstrated *in vitro* in those infections which induce poor immunity to reinfections, it appears that parasites are also able to evade the host immune mechanism that they themselves induce, thus allowing them to survive for long periods in the immune hosts (Soulsby, 1972; Porter and Knight, 1974; Cohen and Sadun; 1976; Ogilvie and Wilson, 1976).

Following an active infection, 3 different categories of immune response can be distinguished.

Sterilizing immunity: This type of immunity is associated with a complete elimination of the parasite and with a life-long specific immunity to reinfection (Porter and Knight, 1974). This situation occurs in certain forms of human cutaneous leishmaniasis, trypanosomiasis in rats and cattle, and rodent malaria. However, the pattern of acquired immunity is not constant for a given parasite but often exhibits wide variation in different hosts. *Plasmodium berghei*, for example, produce sterilizing immunity in rats, but a rapidly fatal infection in mice (Porter and Knight, 1974). The extent to which acquired immunity determines these different responses to the same pathogen has not been adequately investigated.

Nonsterilizing immunity: This is associated with persistence of the parasite in the host at relatively low density (Porter and Knight, 1974). In this type of immunity, established parasites are resistant to the immune mechanisms which are capable of eliminating establishing parasites. There are many suggestions as to how the parasites could survive in the immune host. Among these suggestions are antigenic variations of the parasites, acquisition of host or host-like antigens on their surfaces, or production of factors that counteract the action of the immune components of the hosts (Ogilvie and Wilson, 1976). This situation is consistently observed in a majority of helminthic infections and also occasionally in protozoal infections, e.g., human and simian malarias, trichomoniasis in cattle and coccidiosis in birds.

Absence of effective immune response: Many parasitic infections in human fall into this category and this includes African trypanosomiasis, amoebiasis and many nematode infections (Jarrett and Urquhart, 1971; Cohen and Sadun, 1976).

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Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

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Sterilizing immunity: This type of immunity is associated with a complete elimination of the parasite and with a life-long specific immunity to reinfection (Porter and Knight, 1974). This situation occurs in certain forms of human cutaneous leishmaniasis, trypanosomiasis in rats and cattle, and rodent malaria. However, the pattern of acquired immunity is not constant for a given parasite but often exhibits wide variation in different hosts. *Plasmodium berghei*, for example, produce sterilizing immunity in rats, but a rapidly fatal infection in mice (Porter and Knight, 1974). The extent to which acquired immunity determines these different responses to the same pathogen has not been adequately investigated.

Nonsterilizing immunity: This is associated with persistence of the parasite in the host at relatively low density (Porter and Knight, 1974). In this type of immunity, established parasites are resistant to the immune mechanisms which are capable of eliminating establishing parasites. There are many suggestions as to how the parasites could survive in the immune host. Among these suggestions are antigenic variations of the parasites, acquisition of host or host-like antigens on their surfaces, or production of factors that counteract the action of the immune components of the hosts (Ogilvie and Wilson, 1976). This situation is consistently observed in a majority of helminthic infections and also occasionally in protozoal infections, e.g., human and simian malarias, trichomoniasis in cattle and coccidiosis in birds.

Absence of effective immune response: Many parasitic infections in human fall into this category and this includes African trypanosomiasis, amoebiasis and many nematode infections (Jarrett and Urquhart, 1971; Cohen and Sadun, 1976).

Regardless of the type of the immune response developed, acquired immunity to parasites is, in general, species- and strain-specific. Furthermore, with some parasites, malaria for instance, it is also stage-specific (WHO, 1975; Cohen and Sadun, 1976).

There are a few distinctive features that characterize the humoral immune response in parasitic infections that should be mentioned before discussing the biological effect of antibodies on the parasites. These include:

Hyperglobulinemia: The circulating level of the various classes of immunoglobulins in many parasitic infections is often elevated and this is mainly the result of enhanced immunoglobulin synthesis rather than decreased catabolism (Ogilvie, 1970; Soulsby, 1972; Porter and Knight, 1974; Cohen and Sadun, 1976). It is possible that this is due to a chronic nature of the infection itself or to an extensive tissue migration by some parasites, thereby allowing continuous antigenic stimulation on the part of the host. However, only a small proportion of the elevated immunoglobulins in the circulation has any activity specific for the parasite involved (Porter and Knight, 1974).

Class specificity of the newly synthesized immunoglobulins: In addition to a diffuse hyperglobulinemia which results in an increase of many immunoglobulin classes, some parasites are known to induce elevation of only a certain class of immunoglobulin (Ogilvie, 1970; Porter and Knight, 1974). For example, elevated IgM has been observed in African trypanosomiasis, chronic schistosomiasis and malaria. Such an elevation is rather non-specific as only a small proportion of the IgM produced has antibody to the parasite involved (Ogilvie, 1970). The mechanism responsible for the preferential stimulation of a particular class of immunoglobulin over another is not known. One possibility is that some parasites have extensive antigenic variation and IgM antibody has to be made continuously against new antigens that emerge. It is also possible that these parasites may elaborate factor(s) that interferes with the regulation of the host immune response, e.g., mechanism for the switching over from IgM to IgG production.

Association of enhanced IgE production and eosinophilia with helminthic infections. Some parasites, particularly the intestinal and tissue helminths, induce a marked elevation of IgE (Table 1) that is most frequently associated with eosinophilia. The following observations regarding the relationship between IgE and parasites have been made:

Table 1

Examples of helminthic infections with increased IgE level.

Ascariasis
Capillariasis
Schistosomiasis
Strongyloidiasis
Toxocariasis
Nippostrongylus infection
Hookworm infection

(1) There is a demonstrable increase of total IgE level in parasitized subjects and this returns to normal when parasites are eleminated (Ito *et al.*, 1972; Grove *et al.*, 1974; Ishizaka *et al.*, 1976 a, b).

(2) Only a small fraction of the increased IgE has demonstrable activity to parasites (Ishizaka *et al.*, 1976 a, b).

(3) IgE antibody to parasite appears after the circulating IgE has reached the peak (Ishizaka *et al.*, 1976 a, b; Jarrett *et al.*, 1976);

(4) Appropriately timed infection by some parasites potentiates the production of IgE antibody to unrelated antigens (Bloch, 1973;

Ishizaka *et al.*, 1976 a, b; Jarrett *et al.*, 1976). Such a potentiation is almost always observed in active infection as artificial immunization with the parasite extract has no effect (Soulsby, 1972; Cohen and Sadun, 1976). Exceptions to this statement are *Ascaris* and *Trichinella* extracts (Cohen and Sadun, 1976).

Depending on the species of the animals involved, the potentiation effect is observed only when infection occurs after animals have been sensitized and begun to make IgE. The degree of potentiation usually parallels preexisting IgE level (Cohen and Sadun, 1976).

Although a close association between enhanced IgE production and parasitic infections has been observed in both natural and experimental infections, neither the mechanism of induction nor the action of IgE antiparasite are well understood. From the several hypotheses that have been proposed, the adjuvant-like effect of parasitic antigens is most credible and is consistent with the limited evidence that are currently available (Cohen and Sadun, 1976; Ishizaka *et al.*, 1976 a, b). These parasitic components can act directly on IgE-B eclls, and not on IgG-B cells. On the other hand, they may act on specific T cells thus resulting in a release of soluble factor that stimulates only IgE-B cells. Infection of rats with *Nippostrongylus brasiliensis* is known to induce differentiation and proliferation of IgE-bearing cells in the mesenteric lymph nodes (Ishizaka *et al.*, 1976 a). Nevertheless, it is also possible that these substances may act indirectly on the suppressor T cells which regulate IgE production. These substances however have never been characterized but, in the case of intestinal parasites, it is most likely associated with the metabolic products elaborated by the intestinal phase of the parasites (Jarrett and Steward, 1973). The adjuvant-like effect of these subtances on IgE-B cells is also consistent with the currently available data on the potentiation of the IgE antibody response to unrelated antigens during the course of parasitic infections.

Specific antibody production that is not related to protection: Parasites are antigenically much more complex than other infective agents, not only because they are larger as some are multicellular, but many of them exist in different developmental stages within the same host. Although some of the antigens that exist in different developmental stages may be similar to one another, stage-specific antigens do exist (Cohen and Sadun, 1976). Under appropriate conditions, all of these antigens should be antigenic and induce antibody response that is heterogeneous with regards to its specificity. The serological tests (Table 2) in parasitic infections have not been as useful as in bacterial or viral infections as the former is rather non-specific for the reason just mentioned.

Table 2

Common serological tests used in diagnosis of parasitic infections.

Test	Disease
Complement fixation, agglutination, immunofluorescence	Amoebiasis, Malaria, Leishmaniasis, Trypanosomiasis, Toxoplasmosis, Trichinosis, Schistosomiasis, Filariasis
Immunodiffusion, immunoelectrophoresis	Amoebiasis, Malaria, Trypanosomiasis, Trichinosis
Circumoval precipitation	Trichinosis, Schistosomiasis

The following discussion will be focussed on protective antibodies as they are immediately more relevant to the host-parasite relationship. Knowledge of the immune mechanisms and antigens involved in protective immunity should provide the most satisfactory solutions for the control of some parasitic infections. However, protective antigens have never been completely defined and characterized for any parasites and this make it difficult to understand the mechanisms involved in acquired immunity and almost impossible therefore to design effective immunoprophylaxis against these agents. Due to the fact that only acitve infection, and not artificial immunization with killed parasites, induces resistance to reinfection (Porter and Knight, 1974; Cohen and Sadun, 1976), it appears therefore that the antigens involved are associated with the metabolic activities of living parasites. Instead of using somatic antigens, excretory and secretory products (ES antigens) have been recently used to immunize animals in attempt to induce protective immunity but the results were variable (Richard and Bell, 1971; Kowalski and Thorson, 1972; Richard and Outteridge, 1974; Kwa and Liew, 1977). Improved techniques for the *in vitro* cultivation of parasites will definitely contribute to a progress in designing a safe and effective vaccine for some of these parasitic diseases.

Although information on the nature of the protective antigens is limited, protective antibodies have been frequently demonstrated in the serum of subjects who recovered from active infection (Ogilvie, 1970; Porter and Knight, 1974; Cohen and Sadun, 1976; WHO, 1975). The presence of such an antibody has been demonstrated most often by a classical method of passive serum transfer. Although it is difficult to generalize the finding obtained from the limited results that are currently available, it appears that successful serum transfer (Table 3) is mediated primarily by

Table 3

Immunity conferred by passive serum transfer.

Ascariasis
Malaria
Schistosomiasis
Trichinosis
Toxoplasmosis
Trypanosomiasis
Coccidiosis

IgG class of antibody and is observed more often in cases which are associated with sterilizing immunity. Even in this situation, very often a large quantity of serum is required to give a complete protection, particularly in the case of helminthic infections (Soulsby, 1972; Armour and Dargie, 1974; Porter and Knight, 1974). Nonetheless, it has been reported that if appropriate serum is available, only a small quantity of antibody is required to give complete protection as, for example, in the case of malarial infection in rodents (WHO, 1975). Frequently the quantity of serum required for protection can be reduced if both serum and sensitized cells are used in the transfer, suggesting that both humoral and cellular components are required for protective immunity (Dineen *et al.*, 1973; Maddison *et al.*, 1976; Wakelin and Lloyd, 1976).

What are some of the possible ways that antibodies can damage, interfere with metabolic activities or kill the parasites? It is known that the immune defence could reduce infectivity, motility, reproductive potential, enzyme activity and oxygen consumption of the parasites (Table 4), thereby resulting in retardation of development, reduction of worm burden or complete protection against reinfection (Porter and Knight, 1974). How does the antibody do this? Does it act directly on the parasite or indirectly by creating conditions unfavorable for parasite survival? Does the antibody act alone or require cooperation with other humoral and cellular components?

Table 4

Direct effect of antibodies on parasites.

Biological Effects	Parasites
Neutralization (reduction of infectivity)	*Trypanosoma* sp., Hookworms, *Plasmodium* sp., *Nippostrongylus braziliensis*, *Trichinella spiralis*
Activation (induction of antigenic variation)	*Plasmodium* sp., *Trypanosoma* sp.
Enzyme inactivation	*Trypanosoma* sp.
Immobilization	*Entamoeba coli*
Reduction of reproductive potential	*N. braziliensis*, *Schistosoma* sp.
Interference with migration	*Schistosoma* sp.
Inhibition and retardation of larval development	*Trichostrongylus* sp., *Ascaris* sp.
Interference with growth regulatory mechanism (stunting)	*Schistosoma* sp., *Angiostrongylus cantonensis*
Decreased oxygen consumption	*N. braziliensis*, *Trypanosoma* sp., *A. cantonensis*
Damaging the gut cells	*N. braziliensis*
Change in permeability	*Trypanosoma lewisi*

Antibody alone is known to exert a diverse effect on the parasite (Table 4), although its action may be potentiated by activation of complement (Porter and Knight, 1974). Theoretically all classes of antibodies must be able to react with and damage the parasites directly one way or another, similarly to antitoxic effect in bacterial infections or neutralization of infectivity of viruses. Antibody of different immunoglobulin classes except IgE and IgD have been found on the surface of parasites removed from an immune host (Kemp *et al.*, 1976; Seesee *et al.*, 1976; Befus, 1977). IgE antibody to parasites has never been shown to exert any direct damage to the parasites but accumulating evidence suggest that it may affect the parasites indirectly by creating environments unfavorable for them to live and survive (Jarrett and Urquhart, 1971; Ogilvie and Parrott, 1974; Cohen and Sadun, 1976). Secretory antibody of the IgA class is known to protect mucosa against surface infections by bacteria and viruses (Hereman, 1974). Its action on parasites has never been demonstrated directly although it has been shown that the intestinal secretion of immune host has anti-parasite activity, but whether or not this plays a role in protection is uncertain. Anti-parasite-activity has been demonstrated in the intestinal secretions of *N. brasiliensis*-infected rats or *Coccidia*-infected birds (Cohen and Sadun, 1976; Poulain *et al.*, 1976 a,b.), and in the vaginal secretion of human infected with *Trichomonas vaginalis* (Ackers *et al.*, 1975). It is not unreasonable to expect that SIgA antibody could react with the secreted and excreted products of the parasites and thereby interfering with their food intake and waste elimination. Anchorage of the parasites to the intestinal wall may also be affected by SIgA class of antibody. Only limited information is currently available on IgD and its biological activity and nothing is known about IgD antibody and parasite.

In addition to these direct actions, antibodies can also collaborate with other humoral and cellular components to accentuate the damage induced by the direct mechanism

Table 5

Specific and nonspecific immune effector mechanisms in some parasitic diseases.

Effector Immunoglobulin	Effector Cell	Complement	Biological Effect	Parasite or Disease
All classes	—	–	Neutralization	Malaria
IgG, IgM	—	+	Cytolysis	Trypanosomiasis Schistosomiasis Malaria
IgG, IgM	Macrophage	±	Immune adherence	Trypanosomiasis Malaria
IgG	K cell, Eosinophil	–	Antibody-dependent cell-mediated cytolysis	Schistosomiasis Malaria?
IgE	Mast cell	–	Immediate hypensensitivity	Worm expulsion
—	T cell	–	Cell-mediated cytolysis	Leishmaniasis, Worm expulsion?
—	T cell and macrophage	–	Non-specific cytotoxicity	Malaria Toxoplasmosis

(Table 5; Soulsby, 1972; Porter and Knight, 1974; Cohen and Sadun, 1976). Expulsion of *N. brasiliensis* from the intestine of rats is a good example of a collaboration between antibody and cells. It is generally accepted that the expulsion in this model is a 2-step immunological process involving the sequential action of antibodies which damage the worms and sensitized lymphocytes which cause the actual expulsion of the damaged worms (Jarrett and Urquhart, 1971; Dineen *et al.*, 1973; Ogilvie and Parrott, 1974). It is not known however why antibody-damaged worms are more easily expelled than undamaged worms or how the cells expell these damaged worms. Nevertheless, recent data suggest that animals seemingly lacking potential to produce antibody can still expell the worms from the intestine. The presence in trace quantity of antibody in these animals has never been ruled out.

There are many possibilities as to how IgE antibody might play a role in acquired immunity in parasitic infections (Ogilvie and Parrott, 1974). Some of these include:

(1) **Direct action:** This is by reacting directly with the ES antigens and thereby interfering with their metabolic functions. The IgE antibody may also interfere with the attachment and penetration of the parasites.

(2) **Indirect action:** This may occur as a result of different mechanisms which include the following:

(a) Recruitment of eosinophils: Eosinophils are attracted to the site of infection when eosinophil chemotactic factor of anaphylaxis (ECFA) is released from IgE sensitized mast cells or through activation of the complement system via an alternate pathway (Despommier *et al.*, 1974; Zucker-Franklin, 1974; Kay, 1976). Why eosinophilia occurs or why ac-

cumulation of eosinophils in the tissue is more prevalent in parasitic infections than in bacterial or viral infections are not clearly understood.

Esoinophils may participate in an antibody-dependent cell mediated cytolysis reaction (Butterworth *et al.*, 1976; James and Colley, 1976; Kay, 1976; Jacobson *et al.*, 1977). The action of IgE antibody is to provide environment that would be more favorable for the parasite destruction by IgG antibody and eosinophils. This reaction is more intense when eosinophil-rich leukocyte preparation is used and is nullified in the presence of antiserum to eosinophils (Butterworth *et al.*, 1976; Butterworth *et al.*, 1977). It is possible that eosinophils are attracted to antibody-coated parasites by the receptors on the surface. At least in the schistosome infection in mice, this reaction may play an important role in acquired immunity because elimination of eosinophils from immune rat abrogates its resistance to reinfection (Mahmoud *et al.*, 1975).

(b) Induction of local anaphylactic reaction: This reaction induces non-specific inflammatory response that provide environmental condition not suitable for parasite survival. This "leak-lesion" effect allows protective antibodies to accumulate at the site of infection. The following evidence are consistent with the contention that the local anaphylactic response mediated by IgE class of antibody may have some protective value (Soulsby, 1972; Cohen and Sadun, 1976).

(1) Correlation between the onset of inflammation and worm expulsion.

(2) Inflammation appears quicker, and it is more intense and more prolonged in immune host.

(3) Reduction of the intensity of inflammatory response by cortisone, antihistamine or irradiation prolongs worm retention.

(4) Worm expulsion occurs when intestinal anaphylaxis is induced by unrelated antigen-antibody reaction.

(5) Immediately before and during worm expulsion, there is an accumulation of vasoactive amines and mast cells at the site of worm expulsion.

On the other hand, there are some evidence that are inconsistent with this contention and these include worm expulsion in the absence of IgE antibody and in animals lacking antibody production potential (Jacobson *et al.*, 1977).

These evidence, though still somewhat controversial, favor the idea that IgE antibody somehow plays a role in acquired immunity in some parasitic infections. The inability to induce IgE antibody and acquired immunity with artificial immunization and the ability to induce IgE antibody and acquired immunity following active infection add more weight to the protective role of IgE antibody. Regardless of its role in protection, enhanced IgE antibody production is valuable in immunodiagnosis as the immediate type of skin reaction is useful in providing a presumptive diagnosis for many parasitic infections in human (Table 6).

Table 6

Immediate skin reaction used as presumptive diagnosis of parasitic infections in human.

Ascariasis
Filariasis
Trichinosis
Echinococcosis
Schistosomiasis
Clonorchiasis
Strongyloidiasis
Diphyllobothriasis
Hookworm Infection

These various mechanisms mainly demonstrated by *in vitro* techniques, indicate that

the host immune components can damage the parasites or interfere with their development and survival. Whether or not these mechanisms are effective *in vivo* is not certain because there are many factors that can interfere with these mechanisms. For instance, blocking antibody, soluble antigens, or soluble immune complexes can interfere with the expression of the cell-mediated response to parasites (Ogilvie and Wilson, 1976). Certain parasites may also decrease the phagocytic and killing action of the macrophages or release anti-complementary factor that nullifies the action of complement (Ogilvie and Wilson, 1976).

In summary, there are several possible mechanisms that the antibody, either by itself or in collaboration with other humoral or cellular components, might participate in acquired immunity in parasitic infections. However, it should be stressed that there are many other factors that will influence the outcome of these interactions. Furthermore, many parasites have the ability to evade immune mechanisms, thus allowing them to survive for a long period in the immune host. With these points in mind, it may seem a long way yet before a safe and effective immunoprophylaxis for parasitic infections will be available for human use. Attenuated vaccines using irradiated larvae or chemically treated larvae have been used in some animals and proved to be rather safe and effective (Silverman, 1970; Soulsby, 1972; Cohen and Sadun, 1976; Poulain *et al.*, 1976 b). The ideal preparation for human use is to have purified protective antigen, either of metabolic or somatic origin, available for use as vaccine but this is not yet possible. It has been shown recently however that a purified antigen obtained from somatic extract or metabolic products from *Taenia taeniaeformis* is effective in inducing acquired resistance to infection by this parasite (Kwa and Liew, 1977). It has been shown that malate dehydrogenase and aminopeptidase enzymes from *Ascaris suum* have some protective value when immunized into guinea pig (Rhodes *et al.*, 1965, 1966). Metabolic antigens from *N. brasiliensis* (Poulain *et al.*, 1976 b) *Trichinella spiralis*, *Ancylostoma caninum*, *Dictyocaulus viviparus*, *Trichostrongylus colubriformis* and *Strongyloides papillosus* (Thorson, 1970) could induce a certain degree of protective immunity in experimental animals, but the nature of these antigens is not known as they have never been fully characterized and investigated. Our group has been interested in using *Angiostrongylus cantonensis* as a model system to study acquired immunity to infection by tissue nematode. The limited data that are currently available show that artificial immunization of rats with the *in vitro* culture fluid from adult females is effective in protecting rats from a lethal challenge infection by infective third-stage larvae (Uahkowithchai *et al.*, 1977).

It is hoped that advance in this area of investigation will be forthcoming in the near future as techniques for the *in vitro* cultivation of parasites are improving and investigators with diverse background and experience, e.g., biochemists, immunologists, and parasitologists, collaborate in their effort to solve the same common problem. When this task is accomplished, it will be a great contribution to the well-being of our society, especially for people living in this part of the world where parasitic infections are still prevalent.

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