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Introduction

Almost without exception, a marked cellular response is a characteristic feature of parasitic infections. Even a cursory examination of a supposedly simple reaction will reveal its complexity, and when a more major reaction is examined, one would have the feeling that to undertake an evaluation of it would be an extremely hazardous enterprise. Fortunately, the newer knowledge of the various cell types will now permit a closer study of the functional aspects of the cell response, and it is this aspect which will be emphasized. A familiarity with the morphology and origin of the cells will be assumed.

The chief concern of this review will be the cell types which are closely associated with the immune response of the host, those which respond as a result of it and other forms which make up the acute inflammatory response. No attention will be paid to degenerative changes which occur in parenchymatous cells of organs or proliferative changes in, for example, fibroblasts, but a brief consideration is warranted of the newer knowledge about the change or loss of function of epithelial cells in parasitic infection.

Epithelial Cell Reactions to Parasitism

Hyperplasia of epithelial cells is a common response to parasitism. It is

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seen in the bile duct or pancreatic duct when these are parasitized by helminths such as Fasciola, Clonorchis, Ascaris, Hymenolepis, Stilesia, etc. or by protozoa (e.g. Eimeria stiedae). Similar responses occur in the urinary bladder (poly formation) in schistosomiasis, in the bronchioles and bronchi in lungworm infection and in the gastro-intestinal mucosae in nematode, trematode and sporozoan infections. Such changes are frequently accompanied by a loss of function of various specialized cells or the acquisition of new functions by the cells. For example, in Ostertagia infection in the abomasum of sheep and cattle, there may be a loss of function of the specialized parietal cells which produce hydrochloric acid and the peptic cells which produce pepsinogen, these being replaced by hyperplastic undifferentiated cuboidal cells (49). In the same infection, mucoid metaplasia may occur, and in fact this reaction is not an uncommon response to parasitic helminths, being seen in the bile duct, stomach mucosa, bronchi, etc.

Recent investigations have correlated such changes with the molecular biology of the parasitism. In the Ostertagia situation, the loss of cells with specialized secretory function leads to a rise in the pH of the abomasum and a failure to activate pepsinogen, there is leakage of pepsinogen into the blood and an increased leakage of plasma macromolecules into the lumen. The latter two effects may be due to imperfectly formed cell junctions as a result of the hyperplasia (49).

The functional changes of the rat intestinal epithelium, which becomes hyperplastic in Nippostrongylus braziliensis infection, have been studied by Symons and Fairbairn (99). Under normal conditions there is stated to be a progressive differentiation of the function of epithelial cells as they migrate distally to be shed at the tips of the villi (81). Nippostrongylus braziliensis infection appears to cause an acceleration of this migration leading to an immaturity of the cells with a concomitant loss, or reduction, of the levels of

maltase, alkaline phosphatase and leucine amino peptidase on the cell surface and in the microvilli of the epithelial cells. Comparable hyperplastic changes have been reported in Necator americanus infection (92), these being claimed as contributory causes to impaired absorption of vitamin A, xylose and fat.

Though there is much to indicate that such changes are essentially non-specific, being seen in non-tropical sprue and niacin deficiency, they are associated with cell hyperplasia in the lamina propria, and this in parasitic infections includes infiltration of polymorphonuclear leucocytes, eosinophils, macrophages, plasma cells and lymphocytes, the latter in various stages of transformation.

Local Accumulation of Lympho-reticular Cells

Lymphocytes, plasma cells and macrophages are traditionally associated with immunological functions, and in many instances the local accumulation of them may progress to a definite, and at times, macroscopic focus of lympho-reticular elements which has the general appearance of a lymph node. Such structures frequently develop around a parasite or its larval stage that has been trapped in the tissues. They are seen later in the course of an infection and frequently at a time when immunity (sensu stricto) develops. It is difficult to avoid a conclusion that these lesions are related to the mechanism of immunity, and in fact in many instances there is much to indicate that immunity against a parasite is mediated at a local level. This statement would, of course, not imply that the major antibody producing organs such as the spleen and lymph nodes do not contribute to the picture.

The local nature of the immune response at an organ level is well illustrated in the case of bovine trichomoniasis in which protective immunity appears to be

mediated solely in the uterus and vagina. A similar situation possibly occurs also in Trichomonas vaginalis in man. This implies that antibody-procuding cells are located in the uterine and vaginal walls, and in fact accumulations of plasma cells have been found there (86). Furthermore, it seems that the vagina can produce antibody independently of the uterus and of the general antibody-producing organs (85).

Another example of a locally mediated immune response, with local cell accumulations, occurs with Eimeria tenella of the cecum of the chicken. In this case, however, immunity created in one cecum somehow is transferred to a surgically isolated collateral cecum (43). The mechanism of this is not yet fully understood, though the transfer of immunity does not appear to be mediated by serum antibodies (44). Pierce and Long (86) suggest that the immunity which develops at a second, previously unstimulated site (the second cecum) may be analogous to the second-set homograft rejection reaction (63).

Accumulation of lympho-reticular cells in helminth infections are seen perhaps to their best advantage in such entities as the lympho-reticular broncho occlusive lesions in lungworm disease of cattle which occur around larvae trapped in the tissues or eggs and larvae which have been aspirated into bronchioles and alveoli (51) and also in schistosomiasis with the formation of pseudo-tubercles around immature schistosomes and eggs in the liver.

Lymphoid Hyperplasia in Leishmania Infections

The immunological role of lymphoid cells in Old World cutaneous leishmaniasis seems fairly clear, and this has been documented by Adler (1,2). Following infection with Leishmania tropica, there is local proliferation of macrophages in which the leishmaniae multiply. This continues until the area is infiltrated

with lymphocytes and plasma cells, and when this happens the macrophages cease to proliferate, the population declines and the number of parasites also decreases until such time that they can be demonstrated by culture techniques only. Eventually, the organisms disappear completely and the cutaneous lesion resolves. The sequence of events varies with the individual and in the absence of specific therapy may occupy a period of 3-18 months. Spontaneous cure is followed by lasting immunity to the causative strain, and this may persist for as long as 20 years. Immunity is established only after the lesion has progressed through the series of cellular reactions which result in spontaneous cure(1), and if the cutaneous sore is removed surgically prior to spontaneous cure, the individual remains susceptible to re-infection.

A similar situation occurs with certain forms of New World cutaneous leishmaniasis. The Uta of Peru is usually followed by immunity; however, with muco-cutaneous leishmaniasis, though the initial cutaneous lesion usually heals spontaneously, metastatic lesions occur in the skin, mucosa of the mouth and the cartilages of the nose, mouth and naso-pharynx. A subsequent lymphocytic and plasma cell infiltration may result in a reduction or elimination of the parasites, but in the muco-cutaneous form of the disease chronic metastatic lesions continue to occur. A common feature of these forms of cutaneous leishmaniasis is the lymphoid infiltration, and this is accompanied by a positive Montenegro skin reaction, which is of the delayed type. The reaction develops early in the course of infection and persists for long after spontaneous cure. It can be induced by leptomonads of L. tropica or other species of Leishmania, including those of cold blooded animals, and also antigens from T. cruzi and antigens of T. equiperdum (32).

A type of leishmaniasis in which there is little or no lymphoid cell

invasion nor a positive Montenegro reaction is seen in Leishmaniasis tegumentaria diffusa (20). In this, extensive areas of skin are involved and masses of infected macrophages are found in the dermis, there being no secondary invasion of lymphocytes or plasma cells. It has been suggested that the condition, which has been recorded in a small number of persons in Bolivia, Venezuela and Brazil, may represent a condition of immunological unresponsiveness on the part of the infected person (1). However, there has been no work done to determine whether such persons are genetically deficient or whether the unresponsiveness is one induced by the parasite.

The immunological response to cutaneous leishmaniasis would suggest that it is mediated by delayed hypersensitivity mechanisms. Circulating antibodies are not readily demonstrated in the infection, and there is no evidence that the immunity has an antibody basis.

The situation with visceral leishmaniasis is quite different from that of the cutaneous form. The cellular reactions are similar, being characterized by a massive proliferation of histiocytes and secondary infiltration of lymphoid elements, and these are generalized throughout the spleen, liver, bone marrow and lymphatic glands. However, in most untreated human infections the disease has a fatal ending. Nevertheless, spontaneous cure may occur, and up to 25% of such cases have been reported in India (69) and in Portugal (31). Therapeutic cure of Kala-Azar is followed by resistance to re-infection, and in about 10% of these a local skin lesion (post Kala-Azar dermal leishmanioid) may develop. This lesion contains numerous parasites but they do not become generalized but rather appear to represent a residuum of infection for the maintenance of immunity or premunition.

Whereas delayed skin reactions are common in dermal leishmaniasis, active

cases of Indian Kala-Azar do not show a Montenegro reaction (91), and an absence of the reaction has been noted in Brazilian cases of Kala-Azar (3). A similar situation appears to pertain in the Mediterranian form of the disease. However, a proportion of cases with post-Kala-Azar dermal Leishmanioid do give a positive Montenegro reaction (2). The situation appears different in the East African form of Kala-Azar: positive Montenegro reactions have been observed in treated infections and in 95 of 119 normal volunteers inoculated with a gerbil train of Leishmania that localizes in the dermis (2).

The functional value of the lymphoid hyperplasia in untreated visceral leishmaniasis is difficult to evaluate. Basically, the response fails to contain the infection, but there are indications that the vigor of the lymphoid response may have some value to prolonging life in man and dog. On the other hand, spermophils are very susceptible to infection and show excessive macrophage proliferation with less lymphoid cell infiltration than man or dog. In the spermophil the spleen may be converted to "a nearly solid histiocytoma" (2).

Specific antibodies for Leishmania are present in visceral leishmaniasis, having been detected by complement fixation techniques, either by the use of leishmania antigens or by extracts of mycobacteria, the former being more satisfactory for this (18). There is, however, little correlation between the elevated levels of gamma globulin and the development of immunity or between the complement fixation tests and immunity.

Lymphoid Hyperplasia in Theileria Infections

The Theileria genus occurs in ruminants, and though a detailed consideration of the cell reactions in this infection is not germane to the discussion,

a brief consideration of the immune response serves to illustrate an infection in which immunity, when it does occur, is solid for many years. The important species is Theileria parva, which causes East Coast Fever, a disease which is usually fatal and is characterized by lymphoid hyperplasia followed by exhaustion of the lymphoid tissues and leukopenia (46). Immunity cannot be reduced by splenectomy, and its level is not influenced by the degree of clinical response to the first infection. Antibodies have not been regularly detected in infected or immune animals (9).

In this infection, however, the lymphoid cells appear to play a dual role since as well as a presumed importance in the immune response they also serve as host cells for the parasites. Recent work has shown that the two replicative forms of the parasite, the "macroschizonts" and "microschizonts," behave differently in lymphoid cells (46). The former has been cultivated in bovine lymphocytes in association with baby hamster kidney cells, and in these the organism propagates in the multiplying lymphoid cells but does not destroy the host cell (45). The Theileria organism appears to divide at the same rate as the host cell, the parasitic forms being closely associated with the mitotic apparatus and distributed to daughter cells in late mitosis. This process has yet to be conclusively demonstrated in vivo, but there is, as yet, no evidence for new infection of cells by particles liberated from disintegrated lymphoid cells.

It has yet to be determined whether the parasitized lymphocyte or its clonal descendants can eventually become immunologically competent or whether a separate line of cells is involved in this process.

Lymphocyte Populations and Immunoglobulin Types in Parasitic Infections

It is only recently that efforts have been made to determine the relative

proportions of immunoglobulin containing cells which are found at the local site of an immunological event in a parasitic infection. Recent studies (26) of rabbit tissues during experimental trichinosis have used pairs of immunofluorescent reagents specifically reactive with the γ, μ and α heavy chains and labelled with contrasting fluorochromes (16).

A preponderance of IgA containing cells was found in intestinal sections, but this was high both in normal and infected rabbits and the α chain containing cells made up 80-90% of the immunoglobulin containing cells in normal animals. A comparable finding has been reported for the human intestine (23). A relative increase in IgM containing cells was observed early in T. spiralis infection to be followed by an increase in IgG containing cells late in the infection and after hyperimmunization. The distribution of the immunoglobulin containing cells in the spleen and popliteal lymph nodes differed from the intestinal mucosa, IgA cells representing only 2-10% of the fluorescing cells. Soon after infection, there was a relatively high percentage of IgG cells in relation to IgM cells, but late in the infection and in hyperimmunized animals, the percentage of IgM and IgG cells was usually reversed.

In the diaphragm, following larval encystment, each type of immunoglobulin containing cell was observed, the distribution being similar to that of the spleen and IgM cells being the most abundant class.

The role of IgA cells to immunoglobulin production in the intestinal mucosa and to immunity to parasites in general has yet to be clarified. Crandall et al. (26) failed to observe fixation of IgA immunoglobulin to T. spiralis larvae when sections of diaphragm containing larvae were exposed to various immunoglobulins. Specific staining was obtained only with anti-γ chain reagent.

Interest in IgA antibody has increased recently because of the demonstra-

tion of anaphylactic antibody in rats infected with Nippostrongylus brazilien-sis, monkeys infected with Schistosoma mansoni and in other parasitic infections (76). Though not all the anaphylactic antibody detected in these infections may belong to the IgA type, the occurrence of IgA containing cells at the site of an immunological event, especially when anaphylactic mechanisms are postulated as mediators of the immune response, may indicate that these cell types are important in the response. The situation in N. braziliensis infection may, however, require some reconsideration following work with neonatally thymectomized rats in which strong resistance developed in the absence of high levels of anaphylactic antibody (111).

Effect of Immuno-Suppressive Agents on Immunity to Parasites

The manipulation of the immune response to parasites by immunosuppressive drugs, irradiation, thymectomy and bursectomy has been little studied to date. It should, however, offer an invaluable tool in the analysis of immunity to parasites.

The adrenal steroids have been used in a variety of studies on immunity to parasites, and it has been demonstrated, for example, that the elimination of adult worms of T. spiralis im mice, probably an immune event, can be markedly inhibited by cortisone (19,56). In sheep, infected with gastro-intestinal nematodes, excessive doses of adrenal steroid (prenidsolin) failed to have any effect on the immune status (Soulsby, Unpublished), but chlorambucil markedly affected immunity and allowed a population of inhibited larvae to attain patency within a few days (98). On the other hand, prenidsolin has been used successfully to inhibit the immune elimination of Nippostrongylus braziliensis from the gut of rats (75) and cortisone has been used to overcome "innate" resistance

to such helminth parasites as Litomosoides carinii (14) and Nematospiroides dubuis (27).

The interpretation of the effects of adrenal steroids on immunity to parasites is difficult. These compounds have a wide range of effects on almost every aspect of the immune response [see review by Gabrielsen and Good (39)], and consequently it would be unwise to infer a common basis for the immunity from a common end effect of the drugs.

Total body irradiation has been used to study immunity to Trichinella spiralis infection, and following exposure mice failed to show a significant elimination of their adult worms as compared with control animals (113). As might be expected, the irradiation produced a severe leukopenia, but circulating antibody levels were not markedly altered over the experimental period.

Studies on the effect of bursectomy and thymectomy on immunity to the chicken coccidian Eimeria tenella were carried out by Pierce and Long (86). Chickens deprived of bursal tissue by in ovo treatment with testosterone were successfully immunized against E. tenella even though they failed to produce serum antibodies and showed markedly reduced or undetectable levels of immune globulins. In addition, pyroninophilic cells in the ceca or spleen, and secondary foci in the spleen and cecal lymphoid tissue were also very much reduced in numbers. The inhibition of bursal development is recognized as a major factor in reducing the ability of fowls to synthesize immunoglobulins; nevertheless, such fowls still are able to reject skin grafts (106). The indication is, therefore, that immunity to E. tenella is mediated more by cellular elements than by humoral antibody. Unequivocal evidence that immunity to E. tenella in the chick was dependent on cells derived from the thymus was not obtained, but was probably due to the difficulty of ensuring that all thymic tissue had been removed. In

any case, thymic tissue is present in chicken embryos after 14 days of incubation, and there is the possibility that lymphocytes from the thymus had already been distributed in the body by the time of hatching (83).

The effect of thymectomy on immunity in the rat to N. braziliensis has been reported, and in this work neonatal thymectomy failed to alter the acquisition of immunity to the parasite (111). It is of interest also that thymectomy caused a marked reduction in the level of anaphylactic (PCA) antibody.

Much of the foregoing evidence repeatedly invites the idea that in many cases immunity to parasites is mediated more by "cellular immunity" than classical humoral antibody. There is, indeed, increasing justification for this in respect to some infections, but it should not be forgotten that other entities (e.g. malaria, trypanosomiasis, larval cestode infections, etc.) do appear to depend on humoral factors for the protective immune response. In fact, it should be no surprise to find that a whole range of immune responses occurs to parasitic infection and that the protective devices employed vary from parasite to parasite.

Relationship of Delayed Hypersensitivity (Cell Mediated Immunity) to Immunity to Parasites

In many parasitic infections there has long been an inability, or controversy about the ability, to passively transfer immunity with serum. In cases where this has been achieved, comparatively large volumes of serum have been required and frequently only a moderate degree of passive immunity has been achieved. Local passive transfer of antibody into the skin, with subsequent challenge of the sensitized site with cercariae, has been used to demonstrate serum transfer of immunity in schistosomiasis (77). On the other hand, protec-

tive immunity has been transferred by lymphoid cells in at least two nematode infections in which serum transfer failed to convey immunity. This has been achieved with lymph node cells from guinea pigs infected with Trichostrongylus colubriformis (33,105) and with peritoneal cavity cells with Trichinella spiralis (56). A recent report has indicated that serum or lymphoid cells, or these together, could transfer immunity to Ancylostoma caninum in dogs (64). Larsh (56) has gone as far as to conclude that the mechanisms causing expulsion of adult T. spiralis in mice is mediated by a specific delayed hypersensitivity reaction. Hypersensitivity of the delayed type to larval antigens of T. spiralis has been demonstrated following injection of antigens with Freund's complete adjuvant into the foot pad of guinea pigs (54).

Delayed hypersensitivity is well known in leishmaniasis and is the basis of the Montenegro skin reaction for the diagnosis of muco-cutaneous leishmaniasis. It is of interest to note that the leishmaniae are intracellular parasites of macrophages, and in a recent review of delayed hypersensitivity and microbial infection, Mackaness (60) has stated that "without any known exception, organisms which can survive and multiply within host macrophages cause delayed-type hypersensitivity to ... the microbial antigens." A feature of microbial infections in which delayed hypersensitivity plays a pronounced part is that immunization with killed vaccines (with the exception of Mycobacterium) usually does not lead either to a delayed hypersensitive response or to a marked protection against the challenge infection (60). Living vaccines, on the other hand, produce both of these entities. The similarity between the above situation and that which is seen with a number of parasitic infections is striking, and it would be all too tempting to ascribe many of the difficulties of understanding immunity to parasites, and of helminths in particular, to delayed hypersensitivity phenomena.

There is, however, at present a lack of adequate data to support any such claim though there are indications in several directions of a closer relationship between the delayed type of hypersensitivity and parasite immunity than has been hitherto suspected.

A major factor against such a suggestion might be that skin reactions of the delayed type have not been regularly observed in parasitic infection. However, a delayed skin response is seen in Leishmania, Toxoplasma (38), Trypanosoma cruzi (61) and in the early stages of infection with a number of helminths. It is possible also that the delayed skin reaction has not been searched for, especially when an infection already has a marked immediate type response. A further point is that a skin response may be only one of several manifestations of delayed type hypersensitivity and its absence may imply nothing in respect to the reactions which occur at a cellular level.

Mackaness (60) has suggested that continuing antigenic stimulation is necessary for the induction of acquired cellular resistance. Such a situation could certainly pertain in parasitic, especially helminth, infections where materials may persist in the tissues for several months.

In any consideration of specific cell mediated immunity, the lymphocyte plays a prominant part. Cells which derive from small lymphocytes, pyroninophils or "immunoblasts" (28) are very much in evidence in the lymph nodes draining a skin homograft, the rejection site itself and in lymph nodes draining a site to which a contact sensitizing agent has been primarily applied. If searched for, such cells are also common in the local sites of an immune event in a variety of parasitic infections.

A major problem in the study of delayed hypersensitivity has been, hitherto, the absence of an *in vitro* correlate of the condition. The situation is

rendered more difficult in the field of parasitology because of the lack of suitably defined antigens. Recently, however, several in vitro and experimental in vivo systems have been suggested as in vitro correlates, and these include the inhibition of migration of macrophages from capillary tubes, the transformation of small lymphocytes to active blast forms by soluble antigen (or homologous or heterologous lymphocytes) and the disappearances of macrophages from the peritoneal cavity on the injection of antigen. Of less certain significance is the antibody that is cytophilic for macrophages.

A reaction which might, after further study, serve as an in vitro correlate of immunity in helminth infections is one in which pyroninophilic lymphoid cells become strongly adherent to the antibody sensitized surface of helminth larvae.

Interaction Between Pyroninophils and Parasites

Original studies on this system were concerned with the in vivo interaction of Ascaris suum larvae with cell populations in the peritoneal cavity of immune rabbits (95). It was found that within a few hours (1-4), third stage larvae became covered with a mass of cells, and these, when stained, were seen to be a mixture of cells with a distinct pyroninophilic cytoplasm and eosinophils. The reaction could be followed in vitro with a peritoneal cell exudate induced by a bland oil. Adherence of cells to larvae was rapid and firm. An essential requirement for the reaction was that the larvae must have been previously sensitized with antibody, while treatment of larvae with normal serum failed to induce the reaction. The most reactive cell populations were those which contained a high proportion of lymphoid cells, while exudates which consisted principally of macrophages produced poor reactions or no reactions at all. Since the major

reacting cell appeared to be of the lymphoid origin, the reactivity of cells from various lymphoid organs was examined.

Cell suspensions in Eagles Minimal Essential Medium (MEM) plus 5% normal rabbit serum were prepared from popliteal and mesenteric lymph nodes and the spleen of normal rabbits and from rabbits immune to A. suum. Lymphocytes from lymph nodes failed to become adherent to the antibody sensitized surface of A. suum third stage larvae, and when such lymphocytes were exposed to anti-Ascaris suum serum, they similarly failed to adhere to larvae.

Slight adherence of cells was seen with splenic cells from immune rabbits but not with cells from normal rabbits. The cell adhesion was of a low order, however, and not comparable to that observed with peritoneal cell exudates. Exposure of spleen cells to anti-Ascaris serum failed to increase the degree of adhesion or cause adhesion with spleen cells of normal rabbits. A more detailed study of the reactive cells in peritoneal exudates suggested they were transformed lymphocytes and further work utilized cultures of peripheral white blood cells stimulated either with phytohemagglutinin (PHA) or Ascaris suum antigen. Cell cultures were prepared from heparinized blood obtained by cardiac punctures and cultured in MEM Spinner medium, with the addition of 20% inactivated horse serum in "French Square" bottles. Each culture consisted of 10^7 small lymphocytes in 10 ml of medium and to each was added either 1% PHA or a total of 0.6 mg of protein of whole adult worm extract of A. suum. Cells were harvested after 1, 2, 3, 4 and 5 days of culture and the cell suspension centrifuged and washed three times in ice cold MEM plus 5% NRS and finally made up to 1/10th (1 ml) of the original volume. Third stage A. suum larvae from culture were washed three times in veronal buffer and then sensitized to varying dilutions of antibody. Following this, they were washed a further three times to remove

unattached and unwanted serum proteins. White cell adherence reactions were examined for by mixing one drop of sensitized larval suspension with one drop of cells on a slide.

White blood cells from one or two day old cultures failed to become adherent to the surface of antibody sensitized third stage larvae. Exposure of such cells to immune serum, with subsequent washing, also failed to induce cell adhesion. Stained samples of the cell suspension showed small lymphocytes, neutrophils, eosinophils and macrophages. Cell suspension from three day cultures contained cells which adhered strongly to the surface of antibody sensitized larvae, and the degree of white cell adhesion increased with cell suspensions from 4, 5 and 6 day cultures. After 6 days of culture, a marked reduction in the number of cells occurred.

Cell cultures aged 3, 4 and 5 days showed a decreasing number of surviving neutrophils and an increasing number of typical transformed lymphocytes or blast cells. These showed an expanded nucleus, distinct nucleoli, a varying sized rim of basophilic cytoplasm and methyl green pyronine staining revealed a markedly pyroninophilic cytoplasm. Stained preparations of larvae with adherent cells showed the cells to be comparable to the blast forms in the culture, possessing a marked basophilic and pyroninophilic cytoplasm. Larvae with adherent cells were fixed and exposed to goat anti-rabbit globulin serum conjugated to fluorescein isothiocyanate (FITC) and examined under ultraviolet light. Strong fluorescence occurred in the cytoplasm of the attached cells. Similar preparations of larvae and cells were exposed to A. suum antigen, and after adequate washing were then exposed to a rabbit anti-A. suum globulin conjugated to FITC. In this case strong fluorescence occurred both in the cells and on the surface of larvae, giving presumptive evidence that antibody to A. suum

occurred both on the surface and in the cytoplasm of the attached cells.

The reaction showed evidence of specificity in that the most marked reactions occurred with cell cultures obtained from rabbits which were strongly immune to A. suum. These had been immunized by repeated subcutaneous injection of infected eggs (94) or by vaccination with cultures of third stage larvae obtained from culture (95). Cultures of cells from rabbits immune to unrelated antigens and stimulated with the appropriate homologous antigen failed, with one exception, to give strong lymphoblast adhesion (see Table 1). The significance of the reactions with cells from rabbits immune to egg albumin (EA) is unclear.

At low dilutions of immune serum, sensitized larvae attracted cells with equal effect from cultures stimulated with either antigen or phytohemagglutinin; however, with increasing dilutions of antiserum the cells from PHA stimulated cultures were less reactive and serum could be diluted to a point where PHA stimulated cells were non-reactive whereas antigen stimulated cells still gave good leukocyte adhesion. Such results might be explained on the basis that the mitogenic effect of PHA stimulated transformation of lymphocytes "committed" to a wide range of antigens, including Ascaris, but the number of cells in suspension specifically committed to Ascaris would be much smaller than in suspensions of lymphocytes stimulated by the Ascaris antigen.

The system was antibody dependent, and the most satisfactory sensitization was achieved with serum from rabbits immune to A. suum. Thus, rabbits which had been immunized repeatedly with A. suum eggs (94) or with antigens prepared from in vitro cultured larvae (95) provided reactive sera, and the increasing ability of serum to sensitize larvae to attract cells could be titrated during the immunization schedule. Normal rabbit serum failed to sensitize larvae, and

minimal sensitization was obtained with sera from rabbits immunized with various tissues of adult Ascaris (e.g. whole worm, cuticle, testes, etc.). No reaction was obtained when third stage larvae were sensitized with immune serum against human blood group A substance, sheep red-blood-cells, fowl-red-blood-cells, Necator americanus or Turbatrix aceti (Table 2). In all these cases, sensitization of the cuticle by antibody could be demonstrated either by mixed-anti-globulin-agglutination (21), mixed agglutination or immune adherence techniques (96).

The complement requirements for the reaction have yet to be fully clarified. Some sera retain their sensitizing ability after inactivation at 56 °C for 20 or 30 minutes, whereas others fail to do so. The reactivity can be partially restored by fresh guinea pig sera and more or less completely restored with normal rabbit serum. Those sera which are not inactivated by healing are, invariably, those which show the greatest sensitizing ability and are still active when inactivated at 56 °C for 60 minutes or when treated with zymosan, ammonia or are absorbed with sheep red-blood-cell stroma sensitized with a rabbit anti-sheep red-blood-cell serum. However, inactivation at 56 °C for 120 minutes greatly reduced or abolished the sensitizing ability. Treatment of sera with 2-mercaptoethanol abolished the sensitizing ability though such sera were still able to sensitize the surface, as determined by the mixed antiglobulin agglutination reaction. Samples of reactive sera were electrophoresed in agar, and various fractions were eluted from blocks cut from the electrophoretic run. Sensitizing ability for cell adhesion was found to lie in the region where one would expect the macroglobulin fraction of serum, but other serum fractions were able to sensitize larvae without causing leukocyte adhesion.

The significance of this reaction with third stage larvae in the in vivo

immune response remains to be determined. The present evidence indicates that with such larvae, the reaction is largely mediated by cells and serum from animals which are immune to the parasite; however, further work is necessary before it can be claimed that the reaction is an *in vitro* correlate of protective immunity. It is of interest, however, that the reaction occurs only when sufficient time has been allowed for the small lymphocytes to be activated from their "resting" state.

The adherence of cells, which at present are provisionally classified as transformed small lymphocytes, to the surface of third stage larvae is one of a range of white cell reactions with parasites. Using the *A. suum* system, it can be demonstrated that cells of the granulocytic series also become adherent to the surface of larvae, but this reaction lacks the marks of specificity which characterize the response with lymphoid cells. The reactions with granulocytes, together with other reports on the inter-reaction between parasites and white cells, will be discussed in the section dealing with neutrophils and eosinophils.

Lymphocyte Transformation by Parasite Antigens

The transformation of small lymphocytes into larger blast forms, able to synthesize DNA and undergo mitosis, can be induced by mitogens such as phytohemagglutinin (73), various antigens to which the donor is sensitive (e.g. tuberculin) (84) and by mixed leukocyte cultures (7). As far as can be determined, there has been little or no work using this technique for parasitic infections, but there would seem to be every justification for study in this direction in view of the frequent lymphoid hyperplasia which is frequently seen in parasitic infection.

Preliminary experiments at the author"s laboratory have indicated that lymphocyte transformation can be induced in rat lymphocytes derived from animals immunized with A. suum and stimulated with an extract of whole adult worm. Table 3 presents some of these preliminary results. Similar reactions have been obtained with peripheral blood lymphocytes of a man sensitive to Ascaris as the result of laboratory contact over many years (Table 4).

The significance of these preliminary findings is as yet unclear. They may indicate that a delayed hypersensitivity component is a part of the immune response to Ascaris infection and this would be in line with certain aspects of the histological picture of the immunity to Ascaris. However, it is certainly not the sole form of hypersensitivity which occurs, since an immediate type of hypersensitivity is also much in evidence (98). Nevertheless, it is possible that both could exist in the same animal, perhaps mediated by different antigens. The system does, however, provide a useful technique for the study of lymphocytes from different sources using small quantities of antigen, and the recent demonstration by Oppenheim et al. (80) that delayed hypersensitivity in the guinea pig is expressed by increased lymphocyte transformation should encourage work with Ascaris in the guinea pig.

Macrophages

Reticulo-endothelial hyperplasia has already been noted in leishmaniasis, the macrophage serving as a host cell for the parasite. Infected macrophages are very active and are able to penetrate into many tissues and appear to exchange material with neighboring cells by means of pseudopodia through which material passes from one cell to another (1). The general failure of the immune response in visceral leishmaniasis has been suggested as due to an equivalent of blockade

of the RE system. However, the macrophages are actively phagocytic, in fact showing increased indiscriminate activity, and this has been presented as evidence against immunologic paralysis (2); however, this may not be a valid consideration since it has shown that the macrophages of rabbits rendered tolerant in neonatal life to BGG can take up antigen and stimulated DNA synthesis of spleen cells of an immunized animal (42). The absence of an obvious defect of recognition is comparable to the results of in vivo studies with bacterial antigens, showing that tolerant animals were able to recognize antigen similarly as immune animals (72).

The phagocytic function of the macrophage is of particular interest in the haemoprotozoa, and it now is clear that erythrophagocytosis is a major factor in the pathology of malaria (114). Extensive erythrophagocytosis of both parasitized and normal erythrocytes is seen in the internal organs in Plasmodium falciparum infection and it has also been observed in Plasmodium berghei in rats (22) and in Plasmodium lophurae in ducklings (62) and chickens (93).

Phagocytosis of abnormal numbers of normal erythrocytes in malaria has been attributed to various immunological entities, both antigen and antibody, including an opsonizing antibody which is said to develop via an autoimmunity mechanism. However, recent studies by George et al. (41) allowed them to conclude that red cell destruction in P. berghei infection was caused by hypersplenism and not an opsonin. It was suggested that circulating parasites, even in low numbers, stimulate the phagocytic capacity of the RE system, which leads to an increased rate of sequestration of blood cells in the spleen, including normal ones, which ultimately produces a cumulative effect of greater splenic function and greater cell destruction. These authors were unable to detect IgG on red cells and presumed the absence of IgM because of the absence of agglutina-

tion and hemolysis. In a consideration of the possible mechanisms of the immunopathology of malaria, Dixon (34) has suggested a mechanism which is based on the formation of antigen-antibody complexes, unrelated to the erythrocytes, which non-specifically adsorb to the surface of red cells with the subsequent fixation of complement. Such cells are then liable to lysis, which would explain intravascular hemolysis and erythrophagocytosis.

Erythrophagocytosis has been claimed as a cause of anemia in several other intracellular parasitoses; for example, Babesia in rodents (90) and Toxoplasma in humans (52).

Phagocytic activity is also seen in helminth infections, especially where tissue destruction occurs. In parasitosis of the lungs, for example, hemosiderin is frequently seen in macrophages but apart from this there is little evidence of a direct action of macrophages on parasitic stages of parasites in vivo, though this is presumed in light of the frequency of macrophages in areas where parasites are, or have, migrated. In this case, it is likely that they are performing a purely phagocytic function without reference to, for example, delayed hypersensitivity mechanisms.

The adherence of peritoneal macrophages to the surface of second stage Ascaris larvae when these are placed in the peritoneal cavity of mice immune to Ascaris has been reported (25), and this finding is in contrast to the results of the author (95), who failed to observe a reaction with second stage larvae of Ascaris in the peritoneal cavity of rabbits immune to Ascaris; however, it is possible that different animal hosts may respond in a different manner.

Cytophilic Antibody

Cytophilic antibody (sensu stricto) was originally described by Boyden (11)

as a globulin component of serum which would become attached to certain cells so that the cells would then specifically absorb antigen. The original description was applied to a class of rabbit antibodies which would attach to spleen cells of rabbit or guinea pig. Another type of cytophilic antibody, apparently distinct from the spleen cell cytophilic antibody, has now been described, this being capable of becoming attached to macrophages but not to most other cells (12). The role of cytophilic antibodies in delayed hypersensitivity has recently been assessed by Nelson and Boyden (70), who conclude that there is as yet no clear cut evidence for a definite role of cytophilic antibody in delayed hypersensitivity.

Preliminary studies on the possible occurrence of cytophilic antibody in Ascaris infection have been made in the author's laboratory. None has been found in the infection, but difficulty has been encountered in obtaining a satisfactory bis-diazotized benzidine linkage of Ascaris antigen to red cells, and radio-labelled antigen has not been investigated.

The possibility of an antibody cytophilic for lymphocytes has been suggested (70) as a result of work by Koprowski and Fernandes (55), who showed that normal lymphocytes, treated with serum of rats immune to guinea pig spinal cord tissue, acquired an affinity for cultures of cells of the brain of puppies.

It would seem worthwhile, in view of the strong association of immunity to certain parasites with cells, especially lymphocytes and macrophages, to examine for the various cytophilic antibodies, especially where the spleen plays an important role in erythrophagocytosis.

Neutrophils

Neutrophils are the characteristic cells found early in parasitic infections,

especially where there is inflammation and tissue destruction, and local foci of neutrophils are frequently seen around dying or dead parasites in various tissues of the body. At times neutrophilia may be massive and may lead to an increase in the pathological changes associated with the infection (29). In experimental infection of the mouse with Fasciola hepatica, the later stage of migration of this parasites in the liver is associated with a massive outpouring of leukocytes into the tracts left by the migrating liver fluke.

The early neutrophil response is usually replaced by one of lymphoid elements and macrophages, and possibly this represents the onset of a specific reaction to the parasite by (?) sensitized cells. There is much to commend this idea since second infections frequently produce an initial lesion commencing at the lymphoid stage rather than at the neutrophil stage.

As with the other cells, the role of the neutrophils in parasitic infections is unclear. They are part of the early response to a parasite and appear before well formed immunologic reactions are in operation. However, they are also seen in certain immune reactions, particularly the Arthus reaction, but in both cases they are a response to inflammation and vascular injury with subsequent sticking of cells to the blood vessel endothelium and them emigration of the cells to the local tissue.

An account of the substances which promote leukocyte emigration has been given by Hurley (48), who suggested that a factor (or factors) of serum was activated by damaged tissue rather than by direct liberation from injured cells.

The role of antibody in the chemotaxis of polymorphonuclear leukocytes (PML's) was demonstrated by Boyden (10), who showed that incubation of antigen-antibody precipitates in rabbit serum caused the serum to become markedly chemotactic to rabbit PML's. Studies with PML's and Ascaris antigens and antibodies

have demonstrated a similar finding (24).

Adhesion of Neutrophils to the Surface of Parasites

In vitro studies of the adhesion of neutrophils to the surface of second stage larvae of A. suum have been in progress at the author's laboratory. Second stage larvae, when sensitized with antibody, readily become covered with large numbers of neutrophils. Stained preparations of antibody sensitized larvae exposed to buffy coat preparations of rabbit blood show the cells to be mainly PML's with a few adherent eosinophils. No pyroninophilia is seen with methyl green-pyronine, and the cells do not show specific fluorescence when exposed to a goat anti-rabbit globulin conjugated with FITC. Larvae sensitized with rabbit serum will attract PML's from both immune rabbits and guinea pigs and also polymorphs from normal animals. The reaction is antibody and complement dependent and is produced with a variety of immune sera; these include those which will cause the adhesion of pyroniophils from peripheral blood culture to third stage larvae, antisera to various fractions of the adult Ascaris worm and to unrelated parasites such as Turbatrix aceti and Necator americanus and even normal sera of bovine, rat and guinea pig will produce the reaction. While the several types of immune serum might be expected to contribute to the reaction, the ability of normal serum to induce the reaction was somewhat unexpected. However, other studies have shown that second stage larvae of Ascaris, unlike third stage larvae, will non-specifically become coated with a serum component and possibly this has the ability to fix complement and cause the adhesion of polymorphs.

Second stage larvae of A. suum show a definite sequence of cell adhesion with immune serum, especially when examined with antigen stimulated peripheral blood cultures after 1, 2, 3, 4 and 5 days of culture. The adherent cells from

one day cultures consist only of neutrophils and eosinophils, but as the cultivation time increases, the adherent cells consist of a mixture of neutrophils with increasing numbers of pyroninophils and finally the majority of adherent cells are pyroninophils with only a few adherent polymorphs. The adherence of pyroninophils to second stage larvae is, as with third stage, mediated by immune serum, and consequently the adherence of PML's to second stage larvae is, to a great extent, non-specific in that normal serum and normal cells equally will partake in the reaction.

Adhesion of blood "leukocytes" to the surface of other parasites has been variously reported. Leukocytes have been observed to adhere to the surface of schistosomes after the infected host has been treated with an antischistosome drug (71), and microfilariae of Loa loa, after treatment of the infection with diethyl-carbarazine citrate, may accumulate in the capillaries of the liver where they are attacked and enveloped by macrophages (15,112). It has been suggested that the drug sensitized the microfilariae to attack by cells of the reticulo-endothelial system.

Reactions between polymorphs and flukes derived from x-irradiated cercariae of F. hepatica have been described by Dawes (30). Flukes weakened by irradiation show adherent polymorphs on the surface, and these cells then penetrate the epicuticle of the fluke and pass into the core of the cuticle causing disruption of the epicuticle. It has yet to be determined whether this reaction is mediated by an antibody mechanism, but there is every likelihood that some serum factor is concerned in the mechanism.

Other examples of the adhesion of leukocytes to parasites include the adhesion of microfilariae to human leukocytes in the presence of serum of an infected person (82), the adherence of Trypanosoma lewisi to leukocytes of immune rats (57),

the adherence of the pathogenic trypanosomes to leukocytes of guinea pigs and rabbits (58). The latter reaction has been used to differentiate different strains of trypanosomes. Eosinophils from patients with tropical eosinophilia have been shown to adhere to the filariform larvae of Strongyloides and Necator and occasionally to microfilariae (8). In this work, cell preparations from persons infected with hookworm or Ascaris were also reactive but those from normal individuals were non-reactive. It is clear, from these various reports, that a variety of granulocytic cells can become adherent to the surface of a variety of parasites, including protozoa. It has not been established in all cases that antibody is concerned in the reactions but it is likely that both antibody and complement are concerned in the majority of them.

The effect of granulocytic cells on living larvae has not been determined. There are no reports of degranulation of the cells on the surface of parasites, but such events have not been searched for and, in all, a large unexplored field is available for study in this direction.

Eosinophils

The eosinophil is probably the cell which is most traditionally associated with parasitic infection, and yet, despite its characteristic appearance, there are no definitive statements available regarding its function or the factors which mediate its accumulation, sometimes in a spectacular manner, in the tissues. Usually, the eosinophil is not part of the early acute inflammatory response, but occurs at a later stage of inflammation when round cells and plasma cells become evident. To some extent, its appearance is an indication of the age of an inflammatory lesion and the frequent association of eosinophilia with allergic disorders, and especially parasitic infections, has led to the presumption that

this cell type is associated with immunity processes.

Amongst the many theories which have been devised to account for the accumulation of eosinophils either in the blood or locally, three have received more attention than others. These are variations on a theme to do with histamine and can be stated a) histamine attracts eosinophils, b) eosinophils contain an antihistamine or c) eosinophils contain histamine (5). A more recent study of eosinophils and eosinophilia (59) has helped greatly to clarify the factors which induce eosinophilia. Evidence has been provided that this is due essentially to antigen antibody complexes: antigen alone, antibody alone or the by-products of an antigen-antibody reaction have little eosinophilotactic activity. If this work is confirmed, it would provide an explanation for the marked accumulation of eosinophils in parasitic infection since in many cases the stimulating antigen(s) (the parasite) can persist for some time in the body. Eosinophils have also been shown to have a phagocytic function, being capable of ingesting antigen-antibody precipitates (4,59,88); this is also associated with degranulation of the cells and it may be a mechanism whereby the antigen-antibody aggregate is inactivated or degraded. A variety of enzymes have been identified from the granules of eosinophils, including cathepsin, β-glucuronidase, nucleases, phosphatases and peroxides (myeloperoxidase, verdoperoxidase), however the exact function of these enzymes is still in doubt. Some evidence of degranulation of eosinophils on the surface of Ascaris larvae has been noted by the author, but this effect has not been studied in detail nor examined for its possible effects on the cuticle of larvae.

The idea that antigen-antibody aggregates provide the eosinophilotactic response is an attractive one; however, there are several reports which indicate the occurrence of eosinophilia in circumstances where the immune response plays

a small part in the mechanism. Arean (6) has shown that the appearance of eosinophils at the site of injected Ascaris eggs was too rapid to be explained on the basis of an antigen-antibody reaction, and marked eosinophilia has been observed in a child with visceral larva migrans but who was agammaglobinemic and showed almost a complete absence of immune globulins in the serum (47). There are many reports, published over the last few decades, which indicate that antigens from several parasites (including Ascaris) will induce eosinophilia after an initial injection (40,104). Early work in this area almost certainly utilized animals (e.g. dogs) which may have been naturally sensitized to ascarids, but even the more recent experiments should be interpreted with care since animals are commonly parasitized with a low burden of "normal" parasites and these may be sufficient to sensitize an animal to a cross reacting antigen.

In a study of eosinophilia to Toxocara canis infection in guinea pigs, Olsen and Schulz (79) showed that the onset and extent of eosinophilia was somewhat dose dependent; guinea pigs receiving the largest dose of eggs (5,000) showed eosinophilia on the second day while those receiving only 50 eggs developed it on the 10th day. Maximum eosinophilia was seen approximately two weeks after infection in all cases. Schultz-Dale tests with antigens from Toxocara eggs showed a dose dependency between the number of eggs given and the time when reactions could be elicited. Maximal eosinophilic responses at about two weeks after infection have also been observed in other nematode infections, e.g. in Trichinella spiralis (89) and Dictyocaulus viviparus in cattle (107).

An interesting aspect of the work by Olsen and Schulz (79) was that though the eosinophilia persisted in infected animals for 28 days, the larvae of Toxocara, as judged by digestion techniques, did not persist in the guinea pigs for more than 14 days.

Support for the idea that eosinophils are antagonists of histamine is obtained from studies on the mast cell, eosinophil and histamine levles in rats infected with Nippostrongylus braziliensis (108). A marked eosinophilia that occurred from 12 days onwards after infection was associated with a marked decrease in the number of tissue mast cells and this was interpreted to indicate that the disruption of mast cells, with the release of histamine, served to attract eosinophils whose function was to remove the excess histamine (108). However, an alternative explanation might be that mast cell degranulation was induced by a mast cell sensitizing antibody and that eosinophils were attracted by the antigen-antigen aggregates resulting from this.

There is little doubt that the role of the eosinophil in parasitic infection requires much more detailed study.

Mast Cells

Earlier studies of the cell response to initial and repeated infections of Nippostrongylus braziliensis in rats demonstrated that connective tissue mast cells (connective tissue basophils) fluctuated in numbers during such infection (100). This has been confirmed by Wells (108), who showed that mast cell numbers in the rat intestine fell markedly about the 15th day of infection and later rose to levels much higher than those prior to infection. It is unlikely to be fortuitous that at the same time these events occur there is also a loss (self-cure) of adult worms of an initial infection of N. braziliensis. The increased accumulation of eosinophils which occurs at the same time has been mentioned previously.

Degranulation of mast cells can be brought about by a number of agencies, including trauma, bacterial toxins, heat, cold, ionizing radiation, etc. (see

Uvnas (102)]. An additional mechanism is the degranulation by an antigen-antibody interaction, and it is now well established that the mast cell plays an important part in the anaphylactic syndrome (68,103). A detailed consideration of the various aspects of this is given by Keller (53). Essentially, mast cell degranulation may occur on contact with antigen after active sensitization and, according to the animal species, after passive immunization with serum. Mota has postulated a "mast cell sensitizing" antibody which is non-precipitating, appears early after immunization and may be present in the blood for only a short time (65,66). The most satisfactory method for its demonstration is by homologous passive cutaneous anaphylaxis, and in the rat its production is much accentuated by the use of Haemophilus pertussis vaccine. Consequently, it closely resembles the "reagin" type of antibody (67).

The occurrence of an antibody similar in nature to "reagin" has been reported to be closely associated with immunity to N. braziliensis in the rat (74,76), the reagin being detected in some rats immediately after the acquisition of resistance and in all rats one week later. High levels of the reagin were stimulated only by infection with living, adult worms and not be vaccination with worm extracts. Rats immune to N. braziliensis undergo severe anaphylaxis on intravenous injection of antigen, and since the gut of the rat appears to be the major shock organ (109), it has been suggested that a local anaphylactic reaction in the gut may be responsible for the termination of an adult worm infection (101).

It has been shown recently that anaphylactic shock induced by an unrelated antigen-antibody system will enhance passive immunity conferred with antiserum. This produces a significant expulsion of worms compared to rats which were only passively sensitized (9A). In this circumstance, it appears that the anaphy-

lactic lesion has increased the passage of immunoglobulin to the lumen of the bowel.

It would be tempting to ascribe this sequence of events to a degranulation of tissue mast cells in the intestinal wall, brought about by an antigen-antibody interaction, possibly with the characteristics of a mast cell sensitizing antibody. Conclusive evidence for this, however, must await further study.

There is ample evidence to indicate that helminth antigens do have a profound effect on mast cells. A principle of Ascaris will degranulate rat peritoneal mast cells (102) and studies with mice infected with Trichinella spiralis have shown that local (subcutaneous) degranulation of mast cells is dependent on prior sensitization with the parasites (13). In the latter studies maximal reactions were obtained one month after infection, using either metabolic or somatic antigens of T. spiralis. Sensitization could be passively transferred to normal mice, though in this case mast cell injury was less than that observed in actively sensitized mice. Mast cell degranulation in skin pouches of mice immunized against Strongyloides ratti has been described (41a); in this case "excretory and secretory" antigens of infective larvae provided more effective than somatic antigens.

Various other reports have indicated an increase in the number of mast cells in the skin of mice infected with Schistosoma mansoni, Hymenolepis nana and Syphacia and in the skin of patients with schistosomiasis and filariasis (36, 37). An increase in the number of bone marrow mast cells (basophils) and an increased release of these and eosinophils into the blood of guinea pigs following injection of Ascaris body fluid has been reported (17). Comparable work using a highly purified polysaccharide of Ascaris muscle, showed that intraperitoneal injections caused infiltrations of primitive hematopoetic elements of the

erythrocytic and leukocytic series into the liver (78).

Globule Leukocytes

These cells have received occasional attention over the last several years, having been noticed especially in parasitic infections of the gastro-intestinal tract of ruminants. Their relationship to parasitism and in particular to the immunological process hitherto has been somewhat unclear since they have been found in the abomasal and intestinal mucosae of both infected and normal animals. Recently, however, a clearer relationship between gastro-intestinal parasitism and the globule leukocyte has been reported, the cell being common in the mucosae of parasitized animals but infrequent in worm-free animals (110). An increase in globule leukocyte numbers has been observed in the intestinal mucosa of rats infected with N. braziliensis, this being marked on the 12th day, and was co-incidental with the self-cure mechanism (110).

The nature and function of the globule leukocyte has yet to be fully clarified. Some have regarded it as comparable to the "Russel body" cell of the plasma cell series since, by light microscopy, it appears very much the same as that cell (35). The Russel body cell has been shown to contain immunoglobulin, and in the work by Crandall et al. (26) such cells showed intense cytoplasmic fluorescence with anti-μ heavy chain reagents. A reexamination of the identity of mast cells and globule leukocytes by Jarrett et al. (50) showed that in rat, sheep and bovine three related cell types occurred which could be differentiated on their staining reactions with toluidine blue and fluorescence with acridine orange. One form of mast cell was commonly found in the lamina propria of the intestine, gastric wall and peribiliary area in F. hepatica infected livers. The ultrastructure of mast cells and globule leukocytes was reported to be similar

but different from that of the "Russel body" plasma cell, and of special interest was the fact that mitotic activity could be detected in mast cells and globule leukocytes. The globule leukocyte was suggested as an end cell of a range of mast cell types being derived from the type of mast cell which was found in the lamina propria of the digestive tract.

If globule leukocytes are, in fact, a form of mast cell, then a function for them could be envisaged in gastro-intestinal parasitism. They could, as for example in N. braziliensis infection, accumulate in the mucosa and under appropriate stimulation release biologically active amines which in turn could alter the permeability of the gut mucosa for larger macromolecules and possibly also for immunologically competent cells.

General Summary

A wide range of cellular reactions are associated with parasitic infections. Some of these are probably non-specific responses to tissue injury and inflammation, whereas others appear to be mediated by an immunological response. Since protozoan and, especially, metazoan parasites are not only complex antigenic entities but may also cause tissue destruction, it is to be expected that the cell response to them will be complex. Perhaps this complexity has been an unattractive prospect to workers in the past, but with an abundance of information now available on the morphology, ultrastructure, physiology and functions of the wide range of cell types which can be found in parasitic infections, there is much less reason now for the field to be neglected.

TABLE 1

Leukocyte adhesion with antibody sensitized A. suum larvae and cultures of blood lymphocytes from rabbits immune to different antigens and stimulated with the homologous antigen.

Antibody dilution used to sensitize larvae	Lymphocytes from rabbits immune to							
	Ascaris	Ascaris	Ascaris	Normal Rabbit	Sheep Serum	Bovine Gamma Globulin	Bovine Serum Albumin	Egg Albumin
1 in 5	+ + +	+ + +	+ + +	+	+	+ +	+ +	+ + +
1 in 10	+ + +	+ + +	+ + +	-	-	+	+	+ + +
1 in 20	+ +	+ + +	+ +	-	-	-	-	+ +
Diluent	-	-	-	-	-	-	-	-

TABLE 2

Leukocyte adhesion with A. suum larvae sensitized with various dilutions of different immune sera and tested with cultures of blood lymphocytes from a rabbit immune to A. suum and stimulated with A. suum antigen.

Antibody Dilution	Larvae sensitized with serum from rabbits immune to							
	Ascaris Vaccinated Eggs	Ascaris Vaccinated Larvae	Ascaris Whole Worm	Ascaris Adult Cuticle	Ascaris Adult Testes	Blood Group A_1	Sheep RBC	Fowl RBC
1 in 5	+ + +	+ +	+	+	+	-	-	-
1 in 10	+ + +	+ + +	-	+	-	-	-	-
1 in 20	+ + +	+ + +	-	-	-	-	-	-
Diluent	-	-	-	-	-	-	-	-

+ + + Strong leukocyte adhesion; + + medium reaction;
+ weak reaction; - no reaction

TABLE 3

Transformation by Ascaris antigen, of human lymphocytes from an Ascaris sensitive person.

ANTIGEN ADDED	DAYS OF CULTURE	SIZE OF CELLS (MICRONS)				Total %age of cells 7μ or more	Total %age of cells 9μ or over
		<7μ	7μ to< 9μ	9μ to <11μ	> 11		
PHA	3	37 (±8.1)	30.25 (±5.2)	20.5 (±4.7)	12.25 (±5.4)	63 (±8.3)	32.75 (±8.2)
PHA	5	9.25 (±3.49)	24.25 (±6.38)	41.5 (±5.41)	25 (±10.17)	90.75 (±3.6)	66.5 (±6.9)
ASCARIS 0.06 mg/ml	3	58.75 (±18.6)	29.5 (±6.1)	10.25 (±7.69)	1.5 (±0.87)	41.25 (±21.5)*	11.75 (±8.39)*
ASCARIS 0.06 mg/ml	5	68.5 (±21.7)	24.25 (±4.6)	6.75 (±1.5)	0.5 (±0.5)	31.5 (±3.3)*	7.25 (±2.0)
ASCARIS 0.006 mg/ml	5	83 (±3.81)	13.0 (±1.87)	3.75 (±1.92)	0.25 (±0.13)	17.00 (±3.75)	4.0 (±2)
ASCARIS 0.006 mg/ml	3	80.5 (±25.49)	17.75 (±3.24)	1.75 (±1.3)	0	19.5 (±4)	1.75 (±1.29)
CONTROL	3	87.75 (±1.47)	9.75 (±0.8)	2.5 (±1.12)	0	12.25 (±1.4)	2.5 (±1.12)
CONTROL	5	81.75 (±26.04)	14 (±2.92)	4.25 (±2.58)	0	18.25 (±3.3)	4.25 (±4.08)

Values represent percentage of cells in each group.
400 cells measured in duplicate cultures for each set.
Figures in parenthesis = Standard Deviation
*Significant difference (P = < 0.01) between test and controls.

TABLE 4

Transformation, by Ascaris antigen, of rat lymphocytes from rats immune to Ascaris suum.

ANTIGEN ADDED	DAYS OF CULTURE	SIZE OF CELLS (MICRONS)				Total %age of cells >7μ
		< 7μ	7μ to <9μ	9μ to<11μ	>11	
PHA	3	55.75 (±2.45)	35 (±5.2)	8.75 (±5.85)	0.5 (±.5)	44.25 (±9.29)
PHA	5	68.25 (±2)	15.75 (±3.3)	7.75 (±3.87)	8.25 (±3.15)	31.75 (±2)
ASCARIS 0.06 mg/ml	3	91.5 (±1.4)	6.5 (±2)	2 (±1.23)	0	8.5 (±2.65)
ASCARIS 0.06 mg/ml	5	88 (±1)	3.25 (±1.7)	6.0 (±1.35)	2.75 (±1.35)	12 (±1)*
ASCARIS 0.006 mg/ml	3	96.25 (±10.9)	2.0 (±1.2)	1.0 (±1.2)	.25 (±.14)	3.25 (±.35)
ASCARIS 0.006 mg/ml	5	93.25 (±1.68)	3.75 (±1.65)	1.75 (±2)	1.25 (±.26)	6.75 (±1.78)
CONTROL	3	97.25 (±1)	2.75 (±1)	0	0	2.75 (±1)
CONTROL	5	96.5 (±1.4)	2.75 (±.7)	0.5 (±.9)	0.25 (±.14)	3.5 (±1.44)

Values represent percentage of cells in each group.
400 cells measured in duplicate cultures for each set.
Figures in parenthesis = Standard Deviation.
*Significant difference (P = < 0.01) between test and controls.

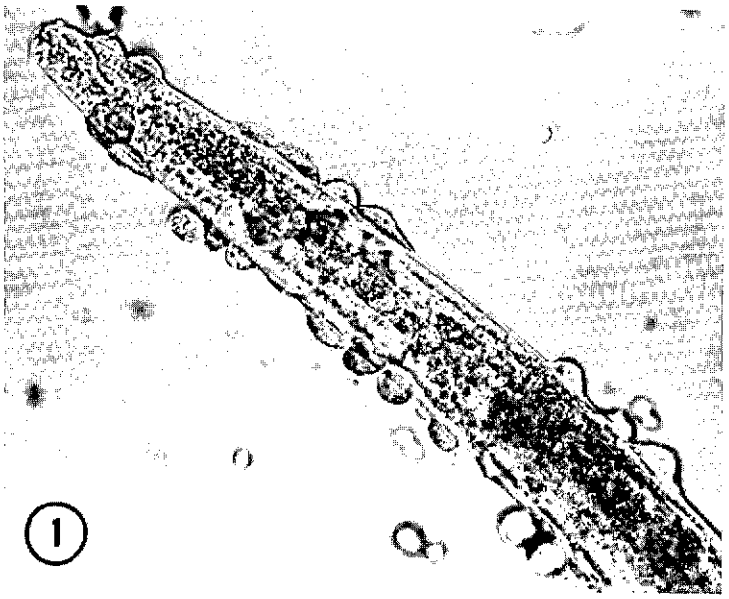
Figure #1: Cells from peripheral blood culture adherent to third-stage Ascaris larvae (x 350).

Figure #2: Pyronine-stain of adherent cells on third-stage Ascaris larvae.

Figure #3: Cells on Ascaris larvae stained with goat anti-rabbit globulin serum conjugated with FITC.

Figure #4: Polymorphonuclear Leucocytes adherent to second-stage Ascaris larvae. Stained Pyronine (x 750).

Figure #5: Polymorphonuclear Leucocytes and pyroninophils adherent to second-stage Ascaris larvae (x 750).



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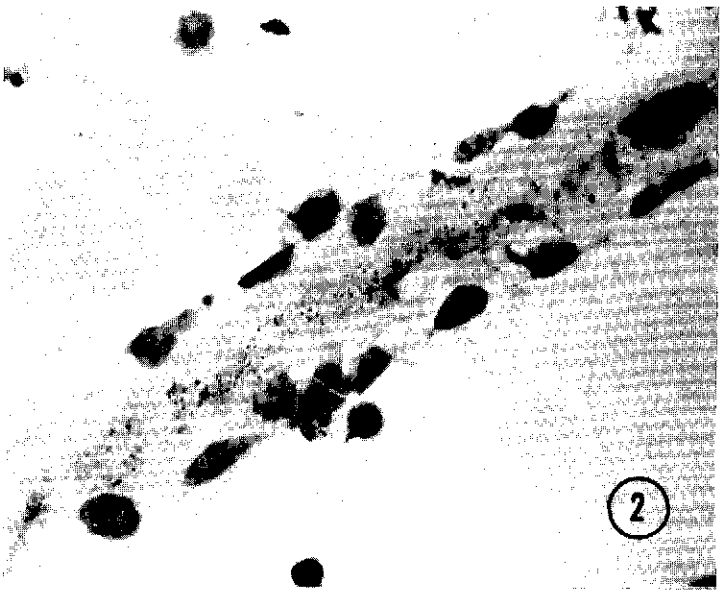

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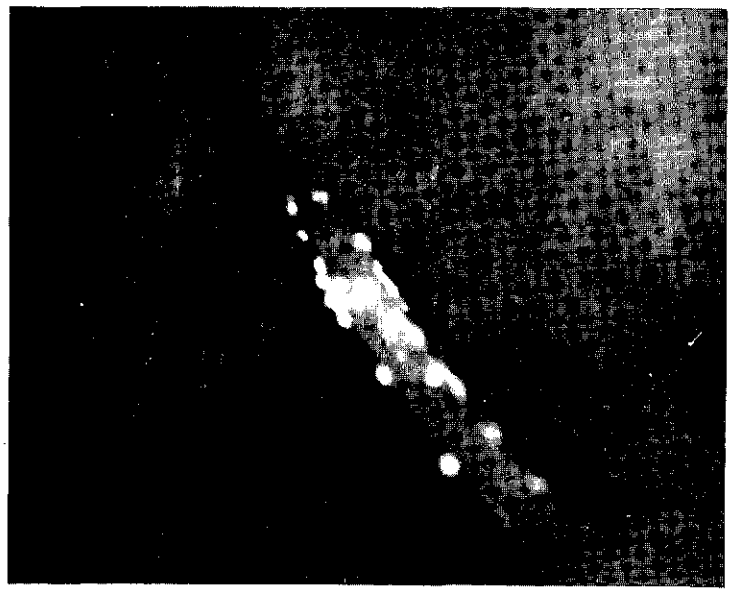

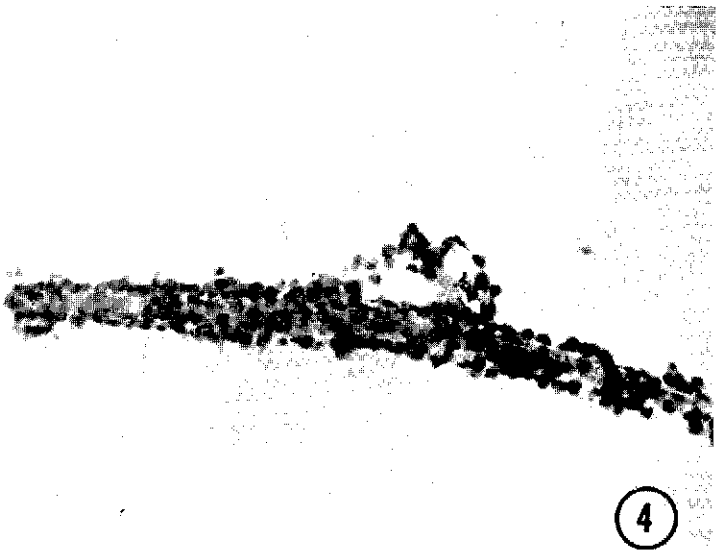

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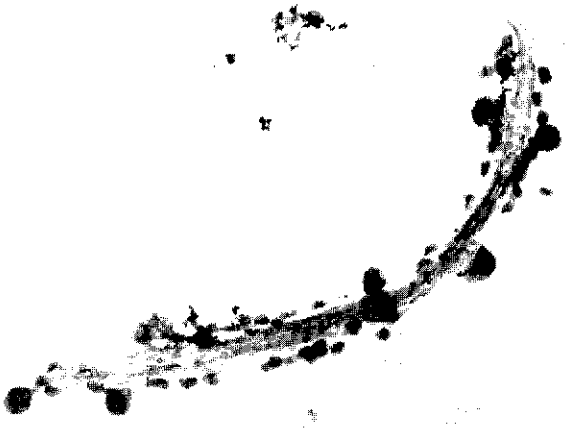

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