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Review

Effector cells, molecules and mechanisms in host-protective immunity to parasites

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A. INTRODUCTION: IMMUNOLOGY AND BALANCED HOST–PARASITE RELATIONSHIPS

Like many infectious organisms with the potential to cause disease, metazoan and protozoan parasites commonly infect their hosts in early, pre-reproductive life. Thus, successful parasites cannot afford to prejudice the survival of large numbers of the susceptible host population and anti-parasite immune responses can be expected to exert an important restraining influence on invading, proliferating, or resident parasites. Immunoparasitology, the study of the immunological aspects of host–parasite interactions is a discipline in a state of expansion and the foundations of experimental immunoparasitology can be found in discussions on the role of the immune system in the evolutionary development of *balanced* host–parasite relationships (e.g. Burnet & White, 1972; Damian, 1978; Dineen, 1978a; Mims, 1976; Smithers, Terry & Hockley, 1969; Sprent, 1959).

In considering immunological events in the interplay between hosts and parasites, it is a matter of some importance to remember that parasites, as well as their hosts, are in a state of evolution; there is every reason to expect as much genetic variation (appropriate to perpetuation of the species) in parasites as in their natural hosts. From this consideration alone, one can anticipate extreme complexity in the immunological aspects of parasitism. For example, it is inconceivable that any one particular anti-parasite effector mechanism could operate in isolation in natural hosts (cf. in unnatural hosts, perhaps) to effect host resistance (see below). The net effect of any one component of the spectrum of immune responses induced by the various life cycle stages of a parasite in its host may be: (1) anti-parasitic (i.e. potentially host protective); (2) pro-parasitic (i.e. potentially parasite protective and counter-productive in terms of host resistance); (3) of no consequence to either host or parasite (i.e. irrelevant other than for immunodiagnosis, perhaps); and (4) harmful to the host (i.e. pathological).

Chronicity is a characteristic of most parasitic infections and persistent antigenic stimulation can itself be expected to influence profoundly the types of immune response induced by resident parasites. Unfortunately for the immunoparasitologist, much of the current knowledge on immune induction and immunoregulation, gained by immunologists in experimental systems, is based on analysis of the events following an antigen pulse where the duration of antigen exposure (at least in high doses) is relatively short. Three aspects pertaining to prolonged duration of infection are of particular interest: (1) the nature of mechanisms of evasion of extant or potentially host-protective immunities utilized by established parasites (Porter &

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Knight, 1974; Ogilvie & Wilson, 1976); (2) the functions and consequences of various accompaniments of chronic parasitic infection such as hypergammaglobulinaemia (literature cited in Cohen, 1976) and immunosuppression (literature cited in Mitchell, 1979) in metazoan and protozoan infections, and the functions and consequences of eosinophilia (e.g. Beeson & Bass, 1977; Butterworth, 1977) and IgE antibody production (e.g. Jarrett, 1973; Dineen, 1978b) in metazoan infections; and (3) the characteristics of specific host responses capable of preventing re-invasion in already parasitized hosts (see below) or of initiating or sustaining the slow process of parasite rejection.

The belief seems justified that an understanding of these aspects of parasitism in clinically and economically important situations, as well as in model systems, will highlight strategies which are, or are not, feasible in immunologically based control of parasitic infection and disease.

Goal-oriented research endeavours in immunoparasitology are concerned with the development of: (1) prophylactic vaccines; (2) immunodiagnostic reagents; (3) means to identify individuals at risk in terms of disease manifestations; and (4) manipulations to reduce the immunopathological consequences of chronic infection. The ultimate output of applied immunoparasitology is, of course, a safe, effective, and cheap vaccine. Theoretically, vaccines may be required to induce immune responses or a state of sensitivity which are, or are not, seen during natural parasitic infection (i.e. the 'natural antigen' versus the 'novel antigen' approach in vaccination). Alternatively, and in situations where immunopathology is a cause of disease, a 'negative vaccine' approach (i.e. desensitization) may be useful. Of importance are the notions that immune responses induced by a vaccine need not cover the spectrum of potential host-protective immune responses (the effects of a limited array of immune responses may tip the balance in favour of the host and enable other types of immune response to be induced more efficiently) nor need they be of such a 'titre' as to prevent *infection* (the anti-parasitic effects of a vaccine-induced state of sensitivity may become operative subsequent to natural infection and may successfully prevent *disease*). The problem areas in the development of parasite vaccines are: (1) identification of relevant antigens and methods for their procurement; (2) identification of relevant immune responses and methods for achieving and maintaining such responsiveness; and (3) identification of individuals who are likely to respond abnormally or suboptimally to the vaccination procedure. These various aspects of parasite vaccines have been discussed (e.g. Clegg & Smith, 1978; Cox, 1978a; Dineen, 1978a; Mitchell, 1979).

Parasites exhibit great structural and life cycle complexity (Cohen & Sadun, 1976). In addition, striking genetically based variation in susceptibility to infection exists in natural hosts (e.g. Wakelin, 1978a; Mitchell, 1979). Thus, and as mentioned previously, no single immunological effector mechanism (e.g. *an* antibody type or *a* host cell type) could be expected to operate in natural hosts, without the involvement of one or more other mechanisms, to prejudice the survival of resident parasites or to ensure host protection against infection. Ogilvie and her colleagues have emphasized this point (see also Sher, 1977) and numerous examples now exist: (1) in intestinal *Nippostrongylus brasiliensis* infections in the natural rat host, the most efficient mechanism of parasite rejection from the intestines involves the operation of both humoral factors (presumably anti-parasite antibodies, but see discussion by Wakelin, 1978b) and cell-mediated inflammatory responses (Dineen, 1978b; Ogilvie & Love, 1974; Ogilvie, Mackenzie & Love, 1977). A similar combination of effector mechanisms appears to operate in rejection of the intestinal parasites, *Trichuris muris* and *Trichinella spiralis* although, in the latter case, cell-mediated immunity may be of principal importance (literature cited in Mitchell, 1979); (2) in the expression of antibody-mediated concomitant immunity in *Taenia taeniaformis* infections in the natural mouse host, IgG isotypes apparently co-operate in mediating resistance to invading larvae (Mitchell, Rajasekariah & Rickard, 1979b); (3) in *Plasmodium knowlesi* infections in rhesus monkeys, antibodies which inhibit merozoite invasion are insufficient for host protection and an unknown splenic activity is also required (Butcher, Mitchell & Cohen, 1978; Langhorne, 1979). Especially with highly adapted parasites such as *Plasmodium* and *Babesia* spp., a combination of immunological effector mechanisms in the expression of net host resistance is predictable and well recognized (literature cited in Brown, 1976; Butcher *et al.*, 1978; Clark, Allison & Cox, 1976; Cohen, 1978; Freeman, 1978; Jayawardena, Targett, Leuchars & Davies, 1978); (4) in the expression of resistance in guinea-pigs to ectoparasitic ticks (e.g. Bagnall, 1975; Wikel, Graham & Allen, 1978) and the intestinal nematode, *Trichostrongylus colubriformis* (Rothwell, 1975) an involvement of T cells, reaginic antibodies, and basophils (± complement) has been implicated (discussed in Askenase,

1977). It can be anticipated that a common statement in the future will be that a particular cellular or molecular mechanism is *necessary* but not *sufficient* for expression of host resistance *at its most efficient*. Another expectation is that the following type of statement will also be more frequent in the future: 'effector mechanisms A and B operate more efficiently in mediating host resistance without the simultaneous interference of responses to infection, C and D'.

The purpose of this article is to review recent findings, interpretations of these findings, and hypotheses which bear on the question of the identity of host cells, molecules, and mechanisms involved in host-protective responses against metazoan and protozoan parasites and, to a lesser extent, ectoparasites. Even after omitting non-immunological responses, and giving cursory attention to those responses which might be called para-immunological (see below), the topic area is still enormous; the central theme will be to highlight systems in which the tools of the 'new immunobiology' are being used in analysis of host-protective immunities and to discuss several systems in which the findings from immunoparasitological research will have an impact on concepts in immunology. [References have intentionally been kept to a minimum and a more comprehensive listing can be found in Mitchell (1979) and other review articles mentioned in the text.] Although the emphasis throughout this review is on balanced host–parasite relationships, it is a fact that numerous important parasitic infections are, biologically, quite unnatural (e.g. the zoonoses in man). The study of abnormal host–parasite situations in the laboratory may or may not be useful even though it is most unwise to try and predict the outcome of any line of biological experimentation. There is no doubt that in the 'totally artificial laboratory model', and particularly in those model parasitic infections which are *uniformly lethal* (yet which are in widespread use), the assessment of relevance is difficult and the generalizing interpretation is to be avoided. One potential output from immunoparasitological analysis of abnormal systems is the identification of host-protective immune responses which function in abnormal hosts but which are actively suppressed or induced inefficiently in natural hosts (discussed in Mitchell, 1979).

B. RESISTANCE TO PARASITIC INFECTION

Immune responses can have a profound anti-parasitic influence as evidenced by the specific effects of vaccination against parasites (e.g. Cox, 1978a) and the numerous examples of transfer of resistance with specifically sensitized lymphoid cells and serum antibodies (e.g. Dineen, 1978b; Phillips & Colley, 1978). Moreover, increased susceptibility to infection in hypothymic nude mice (and reversal in T-cell reconstituted nude mice) is in keeping with a role for T-cell dependent effects in resistance to parasites. In reviewing available information on parasitic infections in hypothymic mice, the gross consequences of exposure to parasites are of two types: (1) increased susceptibility (i.e. increased parasite burdens, increased proliferative rate of parasites, failure to develop resistance, death of the host, or prolonged infection); or (2) comparable susceptibility (or even marginally greater resistance). *All* natural murine parasites are in the first category whereas the second category contains several abnormal parasites, abnormal because the parasite is not usually found in mice or because of laboratory modification of what was once a natural murine parasite. As mentioned above, any parasite which kills the majority of individuals of the host species used in the laboratory can be considered abnormal. The findings using natural parasite systems in nude mice strongly support the notion that T-cell dependent, host-protective immunological responses have been a key ingredient in the evolution of balanced host–parasite relationships.

Notwithstanding the importance of immune responses in host–parasite relationships, resistance to parasitic infections certainly need not have an immunological basis. In the classification of expression of resistance to infection presented in Table 1, the term 'immunity' is used in the broad sense; the categories are *concomitant immunity*, *sterilizing immunity*, *modulating immunity* and *innate immunity* (non-permissiveness). Evidence for concomitant or sterilizing immunity, based on immune responses induced during natural infection, is sought by immunoparasitologists involved in the development of vaccines of the 'natural antigen' type. Greatest difficulties for the vaccinologist are posed by those protozoa in which antigenic variation is likely to be an effective parasite-protective strategy and by parasites which are 'relatively sequestered' (in cells, subcellular sites, or cysts) or 'relatively excluded' (on skin or mucous membranes) from the immune system. If chronicity is a hallmark of clinically and economically important parasitic infections, then sterilizing immunity obviously does not exist in all members of the host species or takes a long time to develop. Concomitant immunity (i.e. resistance to re-

Table 1. Resistance to parasitic infection in vertebrate hosts*

Designation	Alternative terminology	Outcome
Concomitant immunity†	Premunition; Non-sterilizing immunity	Relative or absolute resistance to homologous parasite re-establishment in already parasitized hosts
Sterilizing immunity‡	—	Parasite elimination
Modulating immunity§	—	Parasite modification
Innate immunity¶	Non-permissiveness; Natural resistance	Failure to establish infection

*Principal manifestations are believed to be a block to entry or invasion, direct attack on the parasite or parasitized cell, creation of a hostile local environment, neutralization of parasite-protective mechanisms, and finally, expulsion or death. All categories will have a strong genetic component and varying degrees of species-, stage-, and variant-specific resistance can be expected.

†Commonly seen in balanced host–parasite relationships though readily circumvented by parasite antigenic variation and may be compromised by immunosuppression mediated by established parasites.

‡Difficult to demonstrate since the onus is on the investigator to prove the absence of a single parasite. Expected to be followed by resistance to reinfection. May be confused with innate lifespan of parasite. Broad specificity may be apparent through 'interactive intestinal expulsion' or 'interactive intra-macrophage destruction'.

§Alterations in the parasite include reductions in growth rate, proliferative rate or reproductive rate (including egg production), and changes in migratory behaviour, tissue localization, antigenic constitution, and morphology.

¶As in modulating immunity, there is no reason to assume *ab initio* that immunological responses are involved in the expression of non-permissiveness of the host; the term 'immunity' has been used in its broadest sense in this table.

establishment of the *homologous* parasite in already parasitized hosts) makes 'biological sense' and can be expected to operate against infective or very early forms of the parasite. Thus the search for parasite antigens to be used in a vaccine which simulates concomitant immunity generally centres around the early invasive form of the parasite; of course, this stage of the parasite life cycle need not necessarily be the most convenient *source* of antigen to be used in the vaccine.

In the following section, various immunological and para-immunological mechanisms of host protection against parasites are discussed. The term 'para-immunological' is included simply to cover those anti-parasite effector mechanisms which are unlikely to have any antigenic specificity *and* which may or may not be initiated through classical antigen recognition events and immunological activation mechanisms. For the immunoparasitologist in particular, strict definition of an immunological event is difficult when the final effector molecules in many immune reactions are known to have no antigenic specificity. For example, no difficulties are encountered in labelling, as an immunological effector mechanism, an event such as parasite destruction by an aggressive anti-parasite antibody isotype. The difficulty arises in defining just what is or is not 'immunological' in phenomena such as macrophage activation, mediator production, antibody-independent cellular aggression, chronic inflammation, intestinal sensitivity, etc.

C. EFFECTOR MECHANISMS IN IMMUNOLOGICALLY BASED HOST PROTECTION (Table 2)

1. Macrophage activation

Many of the parasitic protozoa which cause infections of importance in man, survive and proliferate in host macrophages, i.e. *Leishmania* spp., *Trypanosoma cruzi*, and *Toxoplasma gondii*. Such protozoa have

Table 2. Immunological and para-immunological effector cells, molecules and mechanisms in host protection against parasites

Anti-parasite effector mechanism	Candidate effector cells and molecules
1.* *Macrophage activation T-cell dependent inflammation*	Intracellular enzymes and inhibitors
2. Secretory mucosal responses	Inflammatory cells and mediators, goblet cells and mucus
3. Encapsulation	Fibroblasts, collagen
Antibody	
4. Complement fixation	Complement-fixing Ig isotypes
5. Granulocyte focusing	Granulocytophilic Ig isotypes
6. Phagocytosis	Opsonizing Ig isotypes
7. Invasion inhibition 8. Molecular neutralization	Various Ig isotypes including those with relatively inert Fc regions
9. Mast-cell degranulation	Reaginic Ig isotypes
10. *Antibody-independent cellular aggression*	Cytotoxic T cells; NK cells
11. *Soluble non-Ig inhibitors*	Serum components; low mol. wt mediators; lymphocyte, macrophage or granulocyte products

*Number referred to in Section C of the text. Abbreviations: Ig, immunoglobulin; NK cell, natural killer cell of tumour systems; T cell, thymus-influenced (-derived) cell.

obviously evolved means of reducing the innate hostility of the intra-macrophage environment; mechanisms of evasion of intracellular parasitocidal effects include resistance to lysosomal enzymes (discussed in Porter & Knight, 1974) escape from phagolysosomes into the cytosol (Nogueira & Cohn, 1978), and inhibition of fusion of phagosomes (phagocytic vacuoles) with lysosomes (Jones & Hirsh, 1972). Numerous studies with protozoa, and more particularly with intracellular bacteria, have demonstrated that macrophage activation can have broad anti-parasite 'specificity' (see comprehensive discussion in McLeod & Remington, 1977). As a parallel with 'interactive intestinal expulsion' of parasites (see below), the term 'interactive intra-macrophage destruction' may be useful (Table 1). The studies of Behin, Mauel, Biroum-Noerjasin & Rowe (1975) have indicated that the pathogenicity of *Leishmania* spp. in their hosts is related to the ability to grow in activated macrophages (see also Handman, Ceredig & Mitchell, 1979). The reported resistance of the Biozzi Ab/L mice to *Leishmania tropica* (referred to in Biozzi, Mouton & Sant'anna *et al.*, 1979) may be related to a genetically based state of macrophage activation in such mice (Wiener & Bandieri, 1974).

The consequences of macrophage activation by various immunological and para-immunological manipulations include growth inhibition (e.g. Jones, Len & Hirsch, 1975) or destruction (Nogueira & Cohn, 1978) of intracellular parasites. Opsonization of infective forms of the protozoan may have dramatic effects on the intracellular fate of ingested organisms (Jones *et al.*, 1975) although in protozoa a common sequel to Ig binding is rapid shedding of the bound Ig (literature cited in Mitchell, 1979). Whether expulsion of the (compromised?) parasite is an option available to the parasitized phagocyte after activation remains unclear. Other unanswered questions in this area of the immunoparasitology of intra-macrophage parasitism (Trager, 1974) are: (1) the cellular origins (and nature) of soluble mediators which initiate and sustain macrophage activation and attendant parasite destruction (e.g. Handman & Burgess, 1979; Nogueira, 1979); (2) the sequence of intracellular vacuolar events in parasitocidal or parasitostatic (including cross-protective) macrophage activation; (3) the modulation of sensitivity of macrophages to exogenous activators; (4) the precise and preferred locations of various parasitic protozoa in various host macrophage populations; and (5) the candidacy of any parasite-dependent

antigens on the surface of the parasitized cell as targets of aggressive effector cells and antibodies. Indirect evidence has been obtained recently that T-cell recognition of parasite antigens on infected macrophages from a mouse strain which is highly susceptible to *Leishmania tropica*, may be defective because of reduced expression of surface H-2 molecules (Handmann *et al.*, 1979).

2. T-cell dependent inflammation: secretory mucosal responses

Several studies have been performed on the histological aspects of intestinal responses to intra-luminal parasites, and in particular, nematodes (e.g. Miller, Nawa & Parish, 1979; Porter & Knight, 1977; Rothwell, 1975; Ruitenberg & Elgersma, 1976). Although the picture is far from complete, substantial information exists on intestinal responses involving accumulations of mast cells (including the globular leucocytes of some species), eosinophils and goblet cells; all cellular responses demonstrated to date are reduced in parasitized hosts which are markedly deficient in T cells. As in any T-cell dependent response, an important question is whether the reactions are initiated and mediated by T-cell products or via T-cell dependent antibodies. There is some suggestive evidence for a direct cell lineage relationship between T cells and some intestinal mast cells. The intestine appears to convert from a net absorptive organ to a net secretory organ under the impact of various insults and the pharmacological and immunological intricacies of this process have yet to be elucidated. Induction and expression of responses in the intestinal tract will assuredly be complex since florid immune responses to many living and non-living antigenic entities in the intestines would be counter-productive. One essential piece of information which is required is the route by which parasite-derived antigens are transported across the intestinal epithelium (Joel, Laissue & Lefevre, 1978; Owen, 1977; Walker, Cornell, Davenport & Isselbacher, 1972) and the availability of somatic and excretory/secretory/metabolic (ESM) antigens of resident parasites for immune induction as well as for expression of immunological aggression. Also unknown are whether there are 'immunologically privileged' microenvironments in the intestines.

Rejection of parasites from the intestines of mice is a highly T-cell dependent process as judged by persistence of both metazoan and protozoan parasites in nude mice (literature cited in Mitchell, 1979). Rejection in at least some models in which the duration of infection is innately short is achieved most efficiently by the operation of (T-cell dependent) anti-parasite antibodies combined with (T-cell dependent) inflammatory responses in the intestinal tract (see Introduction). The final expulsion phase is mediator-dependent but it is still uncertain whether mediators of intestinal reactions (at physiological concentrations) have important *direct* effects on parasites *in vivo*. In double parasite exposures, the phenomenon of 'interactive intestinal expulsion' (Behnke, Bland & Wakelin, 1977; Bruce & Wakelin, 1977) is well known (Dineen, Gregg, Windon, Donald & Kelly, 1977) as is the phenomenon of protection from rejection of one by the other (i.e. 'interactive protection from expulsion') when one of the participants is *Nematospiroides dubius* (e.g. Behnke, Wakelin & Wilson, 1978). The *N. dubius*/mouse system is one which appears eminently suitable for analysis of the immunological events of *chronic* intestinal parasitism. Although primary infection worm burdens persist for many months, T-cell dependent adult worm rejection and resistance to reinfection can be induced readily in females of some mouse strains but not others by multiple exposures to third stage larvae or following systemic implantation of adult worms (e.g. Day, Howard, Prowse, Chapman & Mitchell, 1979; Mitchell & Prowse, 1979; Prowse, Mitchell, Ey & Jenkin, 1979). A recurring difficulty in dissection of immunity to intestinal parasites is a lack of antigens suitable for use in the detection and measurement of anti-parasite immune responses. Although some progress has been made in the isolation of intestinal nematode antigens (see reviews by Clegg & Smith, 1978; Wakelin, 1978b), the methods available for the quantitative isolation of unmodified antigens, plus assays to assess the mode of action of vaccine-induced host-protective immunity, are at a primitive stage of development. It is for this reason of complexity of the organism that extracellular protozoan parasites such as *Giardia muris* (e.g. Roberts-Thomson & Mitchell, 1979) may offer advantages in terms of antigen isolation for studies on specific host-protective anti-parasite immune responses to intestinally located parasites.

3. T-cell dependent inflammation: encapsulation

In various metazoan parasitic infections in mice, chronic inflammatory responses involving fibrous tissue deposition and localized cellular accumulations are known to be T-cell dependent. Examples are the

granulomas associated with *Schistosoma mansoni* eggs in tissues (Byram & Von Lichtenberg, 1977; Phillips & Colley, 1978; Warren, Domingo & Cowan, 1967), the focal reactions to *Nematospiroides dubius* larvae in the intestinal wall (Bartlett & Ball, 1974; Prowse, Mitchell, Ey & Jenkin, 1978), and the encapsulating responses to *Mesocestoides corti* larvae in the liver (Pollacco, Nicholas, Mitchell & Stewart, 1978). Eosinophils are also prominent in these T-cell dependent reactions and roles for this cell type in tissue repair and anti-inflammatory responses have been postulated (literature cited in Mitchell, 1979). In the *M. corti*/mouse system, at least seven consequences of infection are highly T-cell dependent, *viz.* peritoneal eosinophilia, peritoneal malabsorption, hepatic encapsulation, antibody responses, IgG1 hypergammaglobulinaemia, restrained parasite proliferation, and host survival. Useful information should be obtained from the identification of the subtypes of T cell involved in the various manifestations of chronic inflammatory responses to parasites and their products (Johnson, Nicholas, Metcalf, McKenzie & Mitchell, 1979).

T-cell dependent encapsulation, or walling off, of foreign entities can be expected to have net host-protective effects through sequestration of noxious substances (Von Lichtenberg, 1977; Mitchell & Handman, 1977) or reduction in the proliferative rate of certain tissue parasites (Mitchell, Marchalonis, Smith, Nicholas & Warner, 1977; Pollacco *et al.*, 1978). Such responses, however can certainly be the cause of disease in chronic metazoan parasitic infections (e.g. Grove, 1978; Warren, 1977). One area of investigation is concerned with the mechanisms of modulation of granulomatous reactions to *Schistosoma* eggs in tissues; current interest centres around the role of suppressor T-cell activities although the observations to date can be explained by antibody-mediated inhibition of T cells involved in promoting inflammatory responses (e.g. the T cells of DTH reactions). Immune sera are known to be very effective inhibitors of blast transformation *in vitro* (Oppenheim, 1972) using sensitized cells and parasite antigen preparations (literature cited in Weiss, 1978). Immunosuppression is a common accompaniment of chronic systemic protozoan (and to a lesser extent, metazoan) infection, and the contribution of the more architectural effects of T-cell activation such as lymphoid tissue disruption, are beginning to receive attention along with the more usual interpretations pertaining to triggering of lymphocytes of different subpopulations (discussed in Mitchell, 1979). It is anticipated that this area of immunoparasitology will provide new information on the nature and effects of T-cell products as well as leading to further subdivisions of the peripheral T-cell population.

4. Complement-fixing antibodies

A review of complement- (±antibody-)dependent effects on parasites such as *Schistosoma mansoni*, various larval cestodes and *Trypanosoma* spp. has appeared recently (Santoro, Bernal & Capron, 1979).

Much data is available on the immunological mechanisms of resistance to the larval cestode, *Taenia taeniaeformis*, in mice and rats (literature cited in Lloyd & Soulsby, 1978; Musoke, Williams & Leid, 1978; Mitchell, 1979; Mitchell *et al.*, 1979b). This infection provides an excellent example of antibody-mediated concomitant immunity in which already parasitized hosts are resistant to reinfection and absolute protection can be transferred to naive recipients (including highly susceptible nude mice) with antisera. Whilst no information is available on the nature of antigens responsible for inducation of host-protective antibodies, it is clear that the invading oncosphere and early larvae are susceptible to immune attack. Complement-fixing IgG antibodies are involved in mediating host protection and at least one mechanism of evasion of immunological aggression by the established cystic parasite seems to be the production of anti-complementary activities in the cyst (Hammerberg & Williams, 1978). In mice, a combination of protein A-purified IgG2 and IgG1 fractions from serum of infected mice is most effective at transferring protection to nude recipients. The IgG2 fraction presumably contains anti-parasite complement-fixing antibodies; the role of IgG1 antibodies may be to neutralize anti-complementary activities in the larvae, or through its weak affinity for mast cells, to mediate permeability changes in tissue which in turn facilitate the access of cells or other anti-parasite antibodies (see below). There is evidence, however, that purified IgG1 preparations contain both complement-fixing and non-complement-fixing molecular species (P. L. Ey, personal communication).

Striking mouse strain variation in susceptibility to first infection with *T. taeniaeformis* exists in mice. The rate of appearance of host-protective serum activity (presumably antibody) is reduced in mice of susceptible genotype relative to that in mice of resistant genotype. A race against time may exist for the establishing parasite; the development of protective anti-

bodies by the host is time-dependent as is the development of protective mechanisms by the parasite. In resistant strains of mice the titres of antibodies attained early in infection are presumably sufficient to effect destruction of the early liver larvae (or compromise its subsequent development) before parasite-protective mechanisms are fully functional. In keeping with this explanation for mouse strain variation in resistance, the proportion of eggs which develop into liver cysts is higher at low egg doses *in resistant strains* than at high egg doses. Presumably the increased antigenic stimulation with high egg numbers further accelerates the rate at which host-protective antibodies appear in the critical early stages of infection. The *T. taeniaeformis*/mouse and *T. taeniaeformis*/rat systems are well suited to immunoparasitological studies: host-protective antibodies can be used to isolate 'host-protective antigens', protection against first infection using vaccines consisting of parasite antigen preparations is achieved readily, mechanisms of evasion of host-protective immunity can be dissected, and mouse strain variation in resistance to first infection can be exploited to study mechanisms, as well as the genetics of host-protective immunity. For these reasons, *T. taeniaeformis* may become something of a 'type organism' in metazoan immunoparasitology.

5. Granulocyte-focusing antibodies

Granulocytes of various morphological types have anti-parasitic effects which are antibody dependent. Unprotected young larvae such as the schistosomulae of *S. mansoni* (Butterworth, 1977) and newborn larvae of *Trichinella spiralis* (Kazura & Grove, 1978; MacKenzie, Preston & Ogilvie, 1978) are vulnerable to (IgG) antibody-dependent eosinophil-mediated damage. As in the phenomenon of opsonization and subsequent phagocytosis, complement components can be expected to increase the efficiency of granulocyte binding to antibody-coated larvae but may not be obligatory. Damage to susceptible life cycle stages of the parasite may well result from the action of the products of eosinophil granules (Butterworth, Wassom, Gleich, Loegering & David, 1979b) released onto and present in high concentrations at the parasite surface (McLaren, Mackenzie & Ramalho-Pinto, 1977). Eosinophils bind via antibody to microfilariae (Higashi & Chowdhury, 1970) and to various life cycle stages of *Nippostrongylus brasiliensis* (MacKenzie *et al.*, 1978); other cell types with appropriate Fc receptors will bind, and bound neutrophils and macrophages have anti-parasitic effects in the *S. mansoni*/rat (literature cited in Mitchell, 1979) and *Litomosoides carinii*/rodent systems (Subrahmanyam, Rao, Mehta & Nelson, 1976). In systems where macrophages have been implicated in antibody-dependent killing of infective larvae in natural host–parasite relationships, activated macrophages are more effective than non-activated macrophages (e.g. Chaicumpa & Jenkin, 1978). Do eosinophils have unique properties in terms of parasite damage? At least in the natural host–parasite relationship, *S. mansoni* in man, and the closely related *S. mansoni*/mouse system, the answer to this question appears to be yes (Vadas, David, Butterworth, Houba, Sturrock, David, Herson, Siongok & Kimani, 1979; Mahmoud, Warren & Peters, 1975). Important experiments reported by Weiss & Tanner (1979) bear on the question of the efficacy of antibody-dependent granulocyte adherence in mediating parasite destruction *in vivo*. Using *Dipetalonema viteae* microfilariae in hamsters, these workers have demonstrated unequivocally that the combination of antibodies plus granulocytes destroy microfilariae and some evidence has been provided that IgM anti-cuticular antibodies mediate the effect.

6. Antibody-mediated phagocytosis

Ingestion and destruction of antibody-coated or surface-modified parasites and parasitized cells (by phagocytes including macrophages) is thought to be a common effector mechanism in various protozoal infections (e.g. Cohen, 1978; Hamburger & Kreier, 1975; Mahoney, 1977; Rogers, 1974; Nussenzweig, Cochrane & Lustig, 1978). Phagocytosis by a hyperactive or hyperplastic (e.g. Ferrante, Jenkin & Reade, 1978) mononuclear phagocyte system (RES) may be an important 'final pathway' of parasitized erythrocytes and/or free merozoites in Plasmodium and Babesia infections (Taliaferro, 1949). Surprisingly little quantitative information, however, is available on the phenomenon in an immunoparasitological context (discussed in Freeman, 1978). Thus, the contribution of antibody-mediated removal of infected erythrocytes (via opsonization and/or complement fixation) remains an important unknown in diseases such as malaria and babesiosis. Information is urgently needed on the molecular changes in parasitized erythrocytes at various life cycle stages of the intra-erythrocytic parasites, the quantitative and topographical aspects of antigenic changes (and effects of antibody binding), and the biological activities of anti-red cell

antibodies of various isotypes. The major questions in this area are allied to those concerning the functions of the spleen in haemoprotozoal infections. There is no doubt that definition of splenic functions and the role played by various subpopulations of intra- and extra-splenic phagocytic cells will greatly advance our knowledge of host-protective responses in malaria, babesiosis and trypanosomiasis.

7. Antibody-mediated invasion inhibition

Mechanisms to be considered in this section are inhibition of mucosal and cellular invasion and the discussion is related to that in the next section, antibody-mediated molecular neutralization.

Mucous membrane-located Igs may prevent access of antigen or attachment of infectious organisms to the mucosal epithelium, and the intestine as a barrier to metazoan parasite invasion has received considerable attention. On the question of the efficacy of intestinal Igs of various isotypes, it is important to remember that whilst intra-luminal IgA has certain demonstrated properties which would theoretically increase its potency in mediating anti-parasitic effects, the intestine contains various microenvironments (e.g. the epithelial surface) where other Ig isotypes may be protected from proteolysis and where they may perform important functions. What may be the first demonstration of an IgA-mediated block to entry of a parasite has been reported by Lloyd & Soulsby (1978). These authors have provided compelling evidence that intestinally located IgA *antibodies* can protect young mice against infection with the larval cestode. *Taenia taeniaeformis* . In this same study, IgG, but not IgA, antibodies were shown to be host protective when administered parenterally (see Section C4 above). Although comprehensive histological studies of the type reported by Heath & Pavloff (1975) are required in this system, in all probability hatched and activated oncospheres have been prevented from penetrating the intestinal mucosal barrier in the mice given purified colostrum-derived IgA containing anti-oncospheral antibodies. As discussed above, the *T. taeniaeformis*/mouse and *T. taeniaeformis*/rat systems are proving to be extremely powerful models for the analysis of antibody-mediated, host-protective immune responses.

Inhibition of invasion of red cells by Plasmodium merozoites can be mediated by antibody (Butcher *et al.*, 1978; Cohen, 1978) and the red cell recognition and penetration molecules of merozoites [and hepatocyte recognition structures of Plasmodium sporozoites (Nussenzweig *et al.*, 1978)] are very attractive candidates for species-specific (rather than variant-specific), vaccine-based control of malaria and babesiosis. In all probability, these structures are weakly immunogenic in natural hosts and the titres of antibody obtained even following vaccination with merozoites in powerful adjuvants may be quite low. There are presumably good opportunities here for 'immunological engineering' of isolated recognition structures to increase immunogenicity via linked antigen recognition. An approach being followed by several groups is to determine whether high-titred hybridoma-derived anti-merozoite antibodies are sufficient to protect against Plasmodium and Babesia infections. As emphasized in the Introduction, a combination of effector mechanisms is likely to be required using the 'natural antigen' approach to vaccine-based control of a highly evolved, highly successful, host–parasite relationship. As in immunological approaches to fertility regulation, however, the 'novel antigen' approach offers potential as a strategy in which functional immunity may be induced by relatively simple antigens (discussed in Mitchell, 1979). Of course, the overriding consideration in using 'novel antigens' in vaccines is that of safety since there are usually good reasons why molecules are relatively non-immunogenic, one being the fact that the molecule under consideration is a self antigen.

8. Antibody-mediated molecular neutralization

Enzymes produced and required by metazoan parasites for feeding purposes and tissue penetration have long been considered attractive candidate molecules for immunological intervention (Chandler, 1935). Moreover, enzymes and other molecules associated with growth and development (including moulting) are potential targets (see discussion by Clegg & Smith, 1978). There is currently, however, no example of an antibody, induced by vaccination or natural infection, which has been proved to be host protective because of enzyme neutralization activity.

Theoretically, any parasite molecule which is antigenic and which serves a parasite-protective function may be susceptible to antibody-mediated neutralization or removal. For example, anti-inflammatory or anti-complementary molecules (Hammerberg & Williams, 1978) may be susceptible to immunological neutralization, and a compromised parasite-protective mechanism may increase the susceptibility of the parasite to existing host protective immunities. Toxic and mitogenic molecules of parasites may have immuno-

suppressive effects and host antibodies with reactivity to such entities should indirectly prejudice the survival of the parasite. Again, there is as yet, no example of antibody-mediated neutralization or removal of parasite molecules which has proven host-protective consequences.

Quite speculatively, this category can be extended to include antibody-mediated neutralization of host molecules; neutralization of a host response which is counterproductive in terms of host resistance should be a means of increasing host resistance. 'Blocking antibodies' have been postulated, but certainly not proven, to be involved in protection of established larval cestodes (Rickard, 1974; Mitchell *et al.*, 1977) and in protection of *Trypanosoma lewisi* in the rat (Farrante & Jenkin, 1977). Anti-idiotypic reactivity against such antibodies may diminish the efficacy of this postulated parasite-protective mechanism. Within this category can be included antibody-mediated regulation of immunopathology; cell-mediated immunopathological consequences of infection may be modulated immunologically and antibody is known to be antagonistic to the expression of cell-mediated immunity.

9. Mast-cell degranulating antibodies

An association between reaginic (IgE) antibodies and chronic ectoparasitic and metazoan (but not protozoan) infections has been recognized. A reasonable hypothesis to account for this association is that mast-cell binding anti-parasitic IgE antibodies (or other mast-cell binding Ig isotypes) and products of degranulated mast cells, are involved in the creation of hostile local environments for parasites. The principal initiators and targets of such a response can be expected to be mucous membrane and skin-located parasites. Immediate hypersensitivity reactions in other locations, however, may increase the permeability of tissues and thus the access of anti-parasitic effector cells and molecules (Steinberg, Kimishige & Norman, 1974; Leid, 1977; Musoke *et al.*, 1978). Reaginic antibodies are presumably involved in the dramatic 'self-cure' phenomenon seen in sheep infected with *Haemonchus contortus* (Stewart, 1953) and a role for cutaneous basophil hypersensitivity has been postulated in resistance of guinea-pigs to ectoparasitic ticks (see Askanase, 1977).

As emphasized by Ogilvie & Parrott (1977) reaginic antibodies seem to fall far short of being an indispensable component of the host-protective anti-parasite immune response. Moreover, it is feasible (although entirely speculative) that ectoparasites and mucous membrane-located parasites have evolved mechanisms to utilize the secretory accompaniments of immediate hypersensitivity for their own purposes (e.g. for feeding). Thus, although products of mast-cell degranulation are attractive mediators of ultimate anti-parasite effects, this component of antibody-dependent, host-protective immunity would seem to be relatively inefficient.

10. Antibody-independent cellular aggression

Mechanisms within this category include direct killing by T cells (e.g. cytotoxic T cells) or natural killer (NK) cells. A direct effect of NK cells or T cells on parasites or parasitized cells has not yet been demonstrated. T cells do not damage schistosomulae after binding to the surface (Butterworth, Vadas, Martz & Sher, 1979a) and no demonstration of T-cell-mediated destruction of red cells infected with haemoprotozoa is available. Even if T-cell stimulating antigens exist on parasitized red cells, T-cell recognition will presumably be highly inefficient in those species where associative recognition (MHC) antigens are poorly represented on erythrocytes. An association of low NK cell activity with increased susceptibility to haemoprotozoa in a murine model is worthy of further exploration (Allison, Christensen, Clark, Elford & Eugui, 1979).

11. Soluble non-Ig inhibitors

Considerable interest centres around the mediators of non-specific resistance induced by a variety of immunotherapeutic biologicals, such as BCG organisms, in haemoprotozoal infections (Clark *et al.*, 1976; Brown, 1969; Nussenzweig *et al.*, 1978). Inflammatory cells, including macrophages, are receiving attention as cellular sources of these mediators. Intra-erythrocytic Plasmodium and Babesia organisms are susceptible to intracellular destruction and the anti-parasite effector mechanisms involved, although of unknown nature and with unknown sites of action, presumably underlie the phenomenon of 'parasite crisis' in malaria. There need be no incompatibility between the influence of mediators with no immunological specificity effective in 'crisis reactions' and the high degree of species or variant specificity in natural infection-induced, or vaccine-induced, resistance to disease in malaria. As is well known, the diversity of various malaria infections and the incredible adaptability of

Plasmodial parasites are such that few generalizations in malaria immunology are possible. Each model or human infection seems to have its unique features. Thus, in some, but not all, host–parasite situations, non-specific resistance to malaria is most impressive and is capable of effecting parasite elimination. Using a highly lethal form of the *P. berghei* parasite in mice, a proportion of intact individuals of some but not all strains of mice can be protected with BCG whilst nude mice remain highly susceptible (Mitchell, Handman & Howard, 1978; cf. Clark *et al.*, 1976). Identification of the mediators of 'crisis reactions' in haemoprotozoal infections and dissection of the relationship between endotoxin sensitivity and non-specific resistance (Clark, 1978; Cox, 1978b) are active areas of research. Judging from the difficulties experienced in the characterization of lymphocyte mediators (e.g. lymphokines) over the past 10 years, the projects are assuredly long term.

D. CONCLUDING COMMENTS

Discussions on the mechanisms of host-protective immune responses in parasitic infections cannot be divorced from those on the mechanisms utilized by parasites to evade such responses (Porter & Knight, 1974; Ogilvie & Wilson, 1975). As predicted from the known complexity of the mammalian immune system, strategies adopted by persistent parasites to thwart, subvert or co-exist with potentially sterilizing or damaging host-protective immunities are diverse. All proposed mechanisms fall into three categories—*reduced net parasite antigenicity*, *modification of intramacrophage environment*, and *modulation of host immune responses*—and each category contains up to ten sub-headings (Mitchell, 1979). Analyses of evasion and the host responses being evaded are handicapped by at least three glaring deficiencies: (1) a lack of isolated and characterized parasite antigens with which to measure *specific* anti-parasite immune responses; (2) a lack of information on the precise biological activities of cells and molecules (e.g. the Ig isotypes) with anti-parasite reactivity; and (3) a lack of information on the nature of mediators with no antigenic specificity which affect parasites either directly or indirectly.

A need for parasite antigens is well recognized by immunoparasitologists; without antigens, anti-parasite immune responses other than to the whole parasite cannot be measured. It is in this area of parasite antigen preparation that great hopes are held for monoclonal hybridoma antibodies and recombinant DNA approaches to antigen production. Monoclonal antibodies will find wide and immediate use in the isolation of antigens from crude parasite antigen mixtures prepared ideally in a manner to prevent antigen destruction by endogenous digestive enzymes. It has already been demonstrated that an immunodiagnostic test with *absolute* specificity can be designed using a hybridoma antibody and the crudest of parasite antigen mixtures (Mitchell, Cruise, Chapman, Anders & Howard, 1979a). In any host–parasite relationship, evidence is eagerly sought for antibody-mediated host protection. Using labelled parasite antigens, host-protective antisera can be compared with non host-protective antisera in the search for what have been called 'functional antigens'. Of great importance here are the methods of biosynthetic and extrinsic labelling of parasite antigens and the technologies capable of reducing parasite heterogeneity by separating the various developmental stages of parasites. For example, the fluorescence-activated cell sorter and a fluorescent DNA intercalating dye have been used successfully to isolate Plasmodium and Babesia-parasitized erythrocytes into populations of varying DNA content (Howard, Batty & Mitchell, 1979).

Experimental immunoparasitology is a rich area for collaborative application of the skills of the parasitologist, cell biologist, immunologist and biochemist. The rewards of research are very obvious and a reasonable expectation is that studies on the immunological (and para-immunological) aspects of host–parasite relationships will further our understanding of basic immunological processes. An impact of immunoparasitology on immunology can be expected from studies on the influence of chronicity (yet plasticity) of antigen exposure on immune responses, the roles of activated T cells of various subpopulations especially in the manifestations of chronic inflammation, the biological activities of immunoglobulins of various isotypes, the nature of immunopathology, the relationship between hypergammaglobulinaemia and immunosuppression, the functions of, and heterogeneity in, macrophage, basophil, and eosinophil populations, and finally, the nature of cellular and molecular aggression against living, multicellular, genetically diverse and complex entities. The biological fascination in natural host–parasite situations is that parasites have been selected in evolutionary time on the basis of the ability to partially thwart host aggression without overwhelming the bulk of suscep-

tible hosts. The tools of the 'new biology' including the 'new immunology' will undoubtedly play a central role in the rapid expansion of knowledge of parasites and parasitism and in the development of new means to control parasitic infections and diseases of clinical and economical importance.

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