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Molecular basis of immune response against parasites

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Parasites, both unicellular and multicellular, have evolved many strategies to evade or overcome host immune response. The molecular basis of parasite adaptations and immune-system mechanisms of killing of parasites are subjects of intense study. The best known of parasite adaptations for evading host immune response is surface-antigen variation, the genetic basis of which has been well studied in at least a few parasites. The immune response to parasites has various components, broadly the B-cell-derived antibody and the T-cell responses, and distinct populations of lymphoid cells are involved. The role of proteins coded by the major histocompatibility complex is also important. Recent understanding of the mechanism of antigen recognition has opened up the promising area of the molecular basis of vaccine development.

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A wide range of organisms, some unicellular, others complex multicellular, invade humans and animals and are dependent on the host for their survival. These organisms (parasites) very often cause disease leading to much morbidity and mortality. Many parasites need vectors for transmission, e.g. snails and the tsetse fly transmit schistosomes and *Trypanosoma brucei* respectively. Often development of the parasite proceeds partly in the vector and is completed in the host Some parasites develop in the host and multiply intracellularly: promastigotes of *Leishmania* transform into amastigotes in macrophages, sporozoites of *Plasmodium* become merozoites in hepatocytes.

For over a century biomedical science has tried to understand host parasite relationships for the major human parasites. While several aspects of this complex phenomenon are becoming clear, important questions

still remain unanswered. The challenges they offer are exciting enough to propel parasites to a forefront position in current biological research.

The primary host response to the parasite is mounted by the immune system. However, generating an 'adequate' response is an uphill task for the host's immune system owing to the myriad variations parasites seem capable of generating. Thus diseases with vastly different clinical features are caused by closely related parasites, e.g. visceral and cutaneous leishmaniasis. Localization of specific disease states to different geographical regions suggests that, in many instances, these differences may be due to involvement of different strains or different species of the parasite. Even in the same species antigens may vary at a rapid rate, making it difficult to define epitopes that can be used for generating protective immunity. Different developmental stages of the parasite also display different sets of antigens, and immune response against one developmental stage of the parasite may not recognize other stages. Thus parasites have developed varied means to survive in the host. It is our interest to understand these principles and develop suitable strategies to overcome the escape mechanisms of the parasites. In this review we attempt to summarize current knowledge of parasite immunology, mainly from a molecular point of view, citing examples from the better-studied parasite systems. For more exhaustive analyses, reviews of individual parasites should be consulted.

Cellular basis of immune response

Claman *et al.*[1] first provided the evidence that T and B cells (lymphocytes) interact in the generation of antibody response to sheep red blood cells. Later studies demonstrated that T cells, derived from the thymus, played a major role in helping B cells, derived from bone marrow, to mount the antibody, or humoral, response[2]. The concept of antigen-presenting cells (APC) came from studies of Mosier *et al.*[3], who showed that lymphocytes, separated on the basis of adherent (macrophages) and nonadherent (T and B cells) populations, were incapable of generating an immune response on their own; both populations were required for the response. Later a number of studies showed that macrophages are one of the major APC and that antigens, after endocytosis by APC, must be processed within them before presentation to lymphocytes[4,5]. It is now clear that processed antigen (i.e. a peptide fragment of the antigen) is bound within the cell by a class-II MHC molecule, encoded in the major histocompatibility complex (MHC), and transported to the plasma membrane where it remains associated with the MHC molecule. It is this complex of peptide (antigen) and MHC molecule that T cells interact with. MHC molecules are thus antigen-presenting molecules. That there is intracellular processing of antigen in APC was suspected from experiments that showed inhibition of antigen presentation by agents (like ammonium chloride, chloroquine, etc.) that inhibit lysosomal processing. Detailed experiments have shown that these agents affect antigen presentation by inhibiting intracellular processing of antigen, as opposed to inhibition of steps involved in antigen presentation itself[5].

However, very little is known about the pathway for processing of endogenous polypeptides ('self' antigens and endogenously synthesized viral antigens) *vis-a-vis* uptake and processing of exogenous polypeptides ('nonself' antigens). It is thought that the normal degradation pathway of intracellular proteins is used for processing of endogenous polypeptides as well. As pointed out earlier, the macrophage was the first cell to be identified as APC. Recent studies have shown that a number of other cell types are also capable of antigen presentation. These include dendritic cells, Langerhans cells, Schwann cells, astrocytes and B lymphocytes[6]. It is believed that macrophages, dendritic cells and B lymphocytes are the major APC *in vivo*.

The mechanism of antigen processing may be dependent upon the nature of the APC[7]. While B cells and macrophages can both present native or denatured (processed) antigens, when in high concentration, to antigen-specific T cells, at low antigen concentration the handling of antigen by macrophages and B cells may be different as the antigen may bind to B cells through surface immunoglobulins[8].

It is well known that immunization with a small nonimmunogenic molecule, or 'hapten', conjugated to a highly immunogenic 'carrier' molecule gives rise to a good antibody response against the hapten. A number of studies *in vitro* and *in vivo* have clearly shown that antibody response to a hapten–carrier conjugate is due to cooperation between carrier-specific T cells and hapten-specific B cells. T cells also play an important role in antibody class switching as well as affinity maturation of antibodies[9,10]. Thus T cells can regulate the level, specific isotype and affinity of antibodies against specific antigens, providing an effective defence against viral, parasitic and bacterial pathogens. T-cell populations that suppress immune response to particular antigens in an antigen-specific manner also occur. Inhibition of immune response by antigen-specific T cells was originally suggested by Gershon and Kondo[11]. However, T suppressor (T_s) cells and their role have not yet been well defined. Different subsets of T cells are involved in T-cell–B-cell interactions. It is possible that suppression can be due to inhibition of the differentiation of partially activated B cells by factors secreted by T cells. Conversely, induction of further differentiation of partially activated

B cells by lymphokines is due to the activity of T helper (T_h) cells.

A number of elegant studies carried out in the sixties and seventies demonstrated the presence of immune response (*Ir*) genes within the MHC locus[12,13]. *Ir* genes and their gene products, viz. the Ia antigens, along with class-II MHC molecules, have been implicated in the mechanism of genetic restriction in T-cell–macrophage and T-cell–B-cell interactions[14,15]. Antigen-specific receptors present on T cells (T-cell receptors, TCR) that recognize antigens in the context of specific MHC molecules—hence genetic or MHC restriction—have been identified, and characterized as heterodimers ($\alpha\beta$ or $\gamma\delta$) of disulphide-bonded chains of molecular weight 40,000 to 50,000 (refs. 16, 17). On the other hand, antigen receptors on B cells are mainly monomeric immunoglobulin M (IgM) and IgD molecules, with a hydrophobic C-terminal domain responsible for anchoring the receptor in the membrane of the B cell[18] The antigen receptor on the surface of a given B cell has the same antigen specificity and idiotype as the antibody molecules secreted by the same cell. In the case of T cells a number of cell-surface molecules other than TCR, e.g. the CD3 complex, CD4, CD8, LFA-1, have been implicated in cell activation[17].

Beginning with Raff, and Cantor and Boyse, many successful attempts have been made to define surface markers present on specific cell types and their subsets, which can be used to identify and purify these cells[19]. These studies have shown that T cells are functionally heterogeneous and that the different cell types can be distinguished from each other on the basis of specific cell-surface markers, e.g. CD4 and CD8 antigens, which are present on T_h and T_{cs} (T cytotoxic/suppressor) cells respectively[20]. Moreover, these markers are helpful in deciphering the pathway for differentiation of T cells, i.e. the functional diversification of T cells that takes place independent of antigenic stimulus[21]. Even within the class of T_h cells, two subclasses, viz. T_{h1} and T_{h2} cells, have now been recognized[22]. The subdivision is based not only on the secretion of lymphokines, e.g. interleukin-2 (IL-2) and interferon-γ (IFN-γ) by T_{h1} cells and IL-4 by T_{h2} cells, but also on functional differences[23,24]. In general, T_{h1} cells are MHC-restricted, carrier-specific, and induce antibody response via antigen-specific interaction with B cells. T_{h2} cells stimulate target cells polyclonally by generation of non-specific soluble factors. Class-I MHC molecules are known to be involved in the killing of virus-infected cells by cytotoxic T lymphocytes (CTL). TCR of CTL see processed endogenously synthesized viral antigen along with a class-I MHC molecule in an MHC-restricted fashion[25]. Thus class-I MHC molecules, which occur on the surface of virtually all nucleated cells, primarily present peptides derived from endogenously made self proteins or viral antigens while class-II MHC molecules, expressed in high density on the surface of APC, present peptides derived from endocytosed exogenous (nonself) antigens to T cells.

Although T cells, unlike the B-cell-derived antibodies, do not recognize native antigens in solution but recognize processed endogenously synthesized molecule or antigen, as in the case of viral antigens, or processed exogenous antigen displayed on the surface of APC in association with appropriate MHC molecules, the molecular mechanisms responsible for generation of diversity of both T- and B-cell antigen receptors are remarkably similar[16,18]. Each polypeptide chain of the receptors can be divided into variable (V) and constant (C) regions. The variable region is encoded by a number of different gene segments V, D and J (for heavy chains; just V and J for light chains), which recombine in different combinations to yield specific receptors. Finally it has also been shown that the domain structure of TCR bears structural similarities with that of immunoglobulins and other molecules, like the MHC antigens, involved in immune response.

Senyick *et al.*[26] first showed that T and B cells recognize distinct sites on an antigen. In their studies with glucagon, antibody response was directed towards N-terminal residues 1–17, whereas T-cell activity was directed towards C-terminal sequences. Subsequently, this was confirmed from data obtained from a number of experimental systems, including insulin, hepatitis B virus and myoglobin[27,28]. In the context of T-cell–B-cell cooperation in antibody response how can one explain the fact that T and B cells see different parts of the same antigen? This can easily be explained by the hypothesis of linked recognition, where T cells initially selected on the basis of recognition of macrophage-processed antigen recognize the same epitope on B cells (in their role as APC) after antigen processing, independent of the original epitope recognized by the B cells.

It was pointed out earlier that T cells are involved in the selection of the class of immunoglobulin secreted by B cells for thymus-dependent antigens. In general IgG1, IgG2, IgA and IgE require T cells and/or products secreted by these cells. Detailed studies on the molecular organization of the constant region of immunoglobulin genes have revealed putative 'switch' regions, which allow reorganization of DNA sequences of different constant regions by a combination of 'switch' recombination and deletion[29,30]. By this process it is possible to juxtapose a different constant region, other than CH_μ, immediately downstream of recombined VDJ sequences. The effect of different T-cell-derived lymphokines, along with other polyclonal stimulators, on class switching is being investigated extensively; particularly, the effect of T_{h2}-derived lymphokine IL-4 on mitogen-activated B cells has revealed a preponderance of IgG1 and IgE

production[6]. Molecular analyses have shown that, besides inducing specific class switching, IL-4 also increases transcription of the γ_1 heavy-chain gene.

It was also mentioned earlier that TCR do not recognize native protein antigens but only antigens that have been processed or physically altered (i.e. unfolded, degraded, denatured) and subsequently displayed in association with MHC molecules. Since antigen interacts with MHC as well as TCR, there must be two sites on the antigen: one, the epitope, which interacts with TCR, and another, the agretope, which interacts with MHC molecules. Detailed studies using synthetic peptides derived from pigeon cytochrome *c* and a number of T-cell lines have shown that, for pigeon cytochrome *c*, the epitope is around residue 99 and an agretope around residue 103 (ref. 31). Such analyses have been carried out with several other antigens, including myoglobin and hen-egg-white lysozyme[32]. These analyses have shown that it is possible to define immune response at the molecular level and to use this knowledge to understand host–parasite relations.

Immune response to parasites

One of the major problems in studying parasite immunology is that parasites in general have a complex life cycle and each stage may display a different set of antigens. The majority of parasites are difficult to grow *in vitro* in large quantities. Thus large quantities of purified antigens required to carry out detailed immunological studies can only be obtained by genetic engineering or chemical synthesis. Considerable progress has been made in identification and characterization of immunologically relevant antigens of parasite origin in the last few years, especially in the case of the malarial parasite *Plasmodium*. Much less is known about other parasites.

Antibody response and protection

In several parasitic infections the humoral immune response plays a very important role in limiting and eventually eliminating the parasite. Schistosomiasis is caused by the multicellular helminth parasite *Schistosoma*. The infective stage, being multicellular, has a highly complex antigenic structure. Unlike in the case of protozoan parasites, in the case of *Schistosoma* antibodies have a definitive role to play in protection. The anaphylactic type of antibodies, IgG2a and IgE, in association with macrophages and eosinophils, can cause death of the worms by antibody-dependent cellular cytotoxicity (ADCC) reactions[33] mediated by oxygen radicals. A number of other cell types, e.g. neutrophils and platelets, have also been implicated in immunity[34]. Direct killing of the parasite by antibody via activation of and lysis by complement components has also been suggested on the basis of parasite-killing experiments *in vitro*[35,36]. Cercariae and freshly prepared schistosomulae are highly sensitive to complement. On maturation the schistosomulae become resistant. The acquisition of complement resistance is due to appearance of a complement-regulatory surface protein. Digestion of this protein with trypsin makes the organism sensitive to complement. However, some of these experiments could not be repeated *in vivo*[37].

Since the infective stages are antigenically complex, various strategies have been adopted for identification of potentially protective schistosome antigens. These include generation of monoclonal antibodies that confer protection in experimental animals, mapping of antigens by T-cell Western blotting, and identification of antigens by immune human serum. Several antigens have thus been identified. Sera from animals vaccinated intradermally recognized paramyosin as the major antigen[38]. The protective glycoprotein antigen gp38 induces IgG2a antibodies, and has a carbohydrate epitope also possessed by the snail vector and keyhole limpet haemocyanin[39]. Sera from resistant subjects preferentially react with a 37-kDa major larval surface antigen of *Schistosoma mansoni*. The amino-acid sequence derived from the corresponding gene shows homology with the glycolytic enzyme glyceraldehyde-6-phosphate dehydrogenase[40]. Another enzyme, glutathione *S*-transferase, also induces IgE-mediated protective immunity. Moreover, genetically engineered 26-kDa antigen generates protective immunity in a number of experimental models including nonhuman primates[41]. An 86-kDa polypeptide antigen, p86, of *S. mansoni* was found to be highly homologous to the large heat-shock protein (HSP) of *Saccharomyces cerevisiae* (HSP90) and *Drosophila melanogaster* (HSP83). This was derived from sequence comparison with the cloned p86 gene. The mRNA coding p86 increased in response to heat shock of adult worms[42]. Surprisingly, a 170-kDa antigen present on the surface of schistosome larvae shares a 14-amino-acid epitope with the virion infectivity factor (vif) of the human immunodeficiency virus (HIV). Antibody against this peptide recognizes both schistosomes and HIV and is capable of protecting against schistosome infection in rats[43]. The human antibody response against a protective antigen, SmW68, of *S. mansoni* varied inversely with severity of disease. It is possible that, in this case, increase in parasitaemia may either suppress immune response or block antibody combining sites by leading to formation of immune complexes[44].

Other parasites, for example *Leishmania and Entamoeba histolytica*, may induce substantial antibody response in the disease state without any effect on protective immunity. In these diseases no role has yet been observed for antibodies. It is likely that the

parasites do not allow antibodies to be made against crucial antigens while allowing generation of antibodies against irrelevant antigens. In invasive amoebiasis most of the immune response was found to be directed towards either intracellular or secretory antigens rather than surface antigens[45].

It was shown as early as in 1941 that immunization with UV-irradiated sporozoites leads to protection against avian malaria. Later this was extended to mice and monkeys. The protection was found to be stage-specific, i.e. the immunized animals were protected against sporozoite challenge but not against blood stages of the parasite. In addition, the route and method of immunization influenced the level of protection achieved[46]. With the help of a monoclonal antibody that conferred protection, it was shown that a circumsporozoite (CS) protein may be the protective antigen. Subsequently the protective epitope was identified as the repetitive peptide (asparagine–alanine–asparagine–proline)$_n$ [(NANP)$_n$ in the single-letter code for amino acids], since antibodies raised against this repetitive region blocked entry of sporozoites into cultured hepatoma cells[47]. The mechanism of protection may not involve the effector functions of antibody (ADCC, complement activation, etc.) but may be related to blocking of sporozoite entry into target cells[48].

Detailed analysis of immune response against sporozoite antigen was carried out to develop CS antigen-based vaccines for malaria. From studies in rodents and humans it was found that the antibodies generated against the repetitive epitope of CS protein following immunization or infection confer only limited protection. The sporozoite-induced immunity cannot be transferred by either immune B cells or immune serum. On the other hand, the evidence shows that T cells are involved in protection and in natural immunity to malaria. T cells can transfer immunity from sporozoite-immunized animals[49]. However, antibodies directed against merozoites, sexual stages and zygote/ookinete antigens can confer protection.

Both T_h- and B-cell epitopes responsible for antibody response have been located at the terminal-repeat region of the *Plasmodium falciparum* merozoite antigen Pf155/RESA. Human antibody response against Pf155/RESA blocks erythrocyte invasion by the merozoites. These antibodies are mainly directed against the B-cell epitope near the C-terminal region. Additional T-cell sites have been located near the central-repeat region[50]. Among other merozoite antigens that could be used for immune targeting is a 45 51-kDa cell-surface antigen. Monoclonal antibodies directed against this protein block parasite growth. Detailed analysis has shown that the epitope is located in a tetrapeptide-repeat (serine–threonine asparagine serine, STNS)[51,52] region.

The gene for Pf25, the 25-kDa zygote/ookinete antigen, has recently been cloned. Detailed analysis of this antigen should be forthcoming. Antibodies directed against this antigen prevent maturation and block further transmission[53,54]. Immune response to Pf25 shows less genetic restriction compared to that to other *Plasmodium* antigens like CS protein. Antibodies directed against the sexual-stage-specific 45/48-kDa antigen can also block transmission. However, even in individuals in endemic areas, there is not much immune response against this antigen. This has been attributed to genetic restriction; however, other factors may also be involved[55].

T-cell response

From the previous section it is evident that many parasitic infections do not elicit antibodies that can protect against reinfection. In these cases T cells play a major role in protective immunity. In malaria, results of the first human-vaccine trial using R32tet$_{32}$ (a fusion polypeptide containing NANP repeats of CS protein) showed very poor antibody response. Of 15 vaccinated individuals, 12 developed antibodies, but titres (except in one case) were very low. This may be due to genetic restriction of antibody response to the NANP-repeat epitope in man, which has not been demonstrated, but has been clearly shown in rodent models[49]. NANP repeats elicit T-cell proliferative response only in mice bearing *I-A*^{b} in the *H-2* region. However, other regions of the CS protein stimulate T cells in the (NANP)$_n$-nonresponder strain, e.g. the *I-A*^{k}-bearing strain B10.BR. This led to the search for other T-cell epitopes using overlapping synthetic peptides. Residues 330–343 elicited response in *I-A*^{d}-bearing mice[56]. In an elegant experiment, it was shown that in *I-A*^{k}-bearing mice it is possible to stimulate T cells specific for (NANP)$_n$ with a synthetic antigen synthesized by linking T-cell-epitope-containing peptide with NANP repeats[57].

In a similar fashion, using overlapping synthetic peptides spanning the whole CS protein of *Plasmodium falciparum*, T-cell recognition sites that gave high response in humans were sought. The two peptides that gave the highest frequency of response were peptides 361–380 and 326–345 (Th2R) (ref. 49). The response was found to be similar to that in mice. In this study nearly 40% of the individuals tested did not respond to any of the peptides. In another study the peptide corresponding to amino acids 378–398 induced T-cell proliferative response in the majority of individuals tested from a malaria-endemic area[58].

CS protein has recently been shown to be a target for CTL in experiments that used live recombinant vaccinia virus containing the CS gene cloned in the viral genome. CTL were assayed against mouse L cells

transfected with recombinant CS molecules. The CTL response was found to be genetically restricted to $H\text{-}2^k$ and maximum activity was obtained with the peptide corresponding to amino-acid sequence 368–390 (ref. 59). The observation that IFN-γ plays a very important role in protection further strengthens the idea that cell-mediated immunity is the protective mechanism. Depletion of $CD8^+$ cells *in vivo* using specific antibodies caused loss of immunity. However, no effect was observed when $CD4^+$ cells were depleted[60,61].

Leishmaniasis is caused by a protozoan parasite (*Leishmania donovani* and *L. tropica*). In man the disease shows a wide spectrum of clinical symptoms, from self-healing lesions to chronic, uncontrolled cutaneous or visceral infections. In mice *L. major* mimics a range of disease similar to that in man. Resistant inbred strains, like C3H/HeN, are capable of controlling parasite growth while susceptible strains, like BALB c, develop a chronic disease which may lead to death. The parasites mostly reside within macrophages in the host.

A number of studies over the last decade have shown that immunity to leishmanial infection is associated with protective T cells, which produce lymphokines that activate macrophages, resulting in the killing of the parasites within the host cells[62–64]. A protective role for antibodies has not been demonstrated. In the last few years there have been major attempts to identify and characterize the subsets of T cells involved to better understand the immune response against *Leishmania*. The role of $CD4^+$ T cells in protective immunity has long been recognized. Development of protective $CD4^+$ T cells required immunization with live *Leishmania* whereas immunization with killed parasite led to exacerbation of the disease[65]. Scott *et al.*[66] showed that resistance in mouse is associated with specific stimulation of IFN-γ- and IL-2-secreting T_{h1} cells (a subset of $CD4^+$ cells), whereas susceptibility is associated with IL-4- and IL-5-producing T_{h2} cells. This was confirmed by Heinzel *et al.*[67] and *Holaday et al.*[68]

Several antigens/antigenic preparations have been reported that stimulate T cells. Lipophosphoglycan (LPG) was the first antigen that was shown to induce protective immunity in mice. Further studies showed that the soluble fraction of LPG actually promoted the disease[69,70]. Other major surface antigens, gp63 (a protease) and H61da, have also been reported to provide significant protection against the disease in mice models[71,72]. Using partially purified antigen preparations (fractions 1 and 9), Scott *et al.*[66] showed that, although both fractions stimulated T cells, only fraction 9 could confer protective immunity. Subsequently it was shown that the T cells responsible for the protection conferred by fraction 9 are T_{h1} cells, whereas fraction 1 stimulated mainly T_{h2} cells[73]. Purified gp72, with *Corynebacterium parvum* as adjuvant, also induced 81% protection[74]. Novel adjuvants, for example liposomes containing phospholipid combinations optimized for T-cell priming, have also been used to boost T-cell responses. Promastigote extracts enriched for cytoplasmic antigens in conjunction with liposome adjuvants induced protective immunity. Protection was found to be directly related to the ability to generate IL-3 (ref. 75).

T-cell immunoblotting identified the antigens recognized by T_h clones generated against fraction 9 as polypeptides of around 8–10 kDa. In addition, T_{h1} cells generated after immunization with fraction 9 also recognized a number of other antigens in the molecular-mass ranges 23–35 kDa and 50–60 kDa (ref. 76). Supplementing the immunoblotting technique with the resolving power of two-dimensional gel electrophoresis, Melby *et al.*[77] showed 50 to 70 distinct leishmanial antigens that induce T cells to proliferate and/or secrete IFN-γ. T cells were isolated from patients who had either recovered spontaneously or were cured by chemotherapy[77]. Several laboratories have reported that antigen-specific $CD8^+$ cells are also important in immune clearance of leishmanial parasites. These cells may be involved in granuloma formation associated with killing of parasites within macrophages[78]. Since T-cell immunoblotting shows a number of leishmanial antigens capable of inducing T-cell proliferation, it is likely that different antigens may stimulate different subsets of T cells, although a majority of the antigens may not be protective. What determines which subset of T cells will get stimulated? To answer this question, it is necessary to analyse in detail not only immunity to various antigens but also the nature of immunization, the adjuvants, etc., which could also influence the generation of particular T-cell subsets[79]. The experiments of Muller and Lewis[65] indicate that protective antigens are associated with live organisms and may possibly be secreted products of the parasite within macrophages.

IFN-γ may be the major lymphokine involved in resolution of leishmaniasis. $CD4^+$ T cells capable of producing IFN-γ on antigenic stimulation *in vitro* have been isolated from localized cutaneous lesions or after cure of visceral leishmaniasis. In experimental visceral leishmaniasis IFN-γ has been implicated in granuloma formation and host resistance[79]. On the other hand, IFN-producing T cells could not be demonstrated in active disease[80]. It is likely that parasite components could suppress IFN-γ production and thereby bypass the immune system of the host. Inoculation of human monocytes with *L. donovani* produces such a suppressor factor (a low-molecular-weight protein), which can influence IFN-γ-induced monocyte killing of intracellular parasites[81]. Both IFN-γ and $CD8^+$ cells have also been implicated in protective immunity against *Trypanosoma cruzi*.

Requirement of T cells in protective immunity against schistosomiasis was demonstrated in experiments using genetically athymic nude mice. Marked reduction in immunity was observed in these animals. $CD4^+$ T cells are thought to be involved (along with humoral immunity) in protection[82]. Immune suppression specifically induced by the infection may be one of the mechanisms by which the parasite ensures its own survival. Macrophages isolated from the granulomas could induce unresponsiveness in $CD4^+$ cytochrome *c*-specific *I-E*^{k}-restricted T cells, showing that schistosome infection may lead to a general immune suppression[83].

Antibody-independent cytotoxicity of human monocyte-derived macrophages towards schistosomulae of *S. mansoni* has been demonstrated by James *et al.*[84] IFN-γ is required to activate the macrophages and the killing involves arginine-dependent production of reactive nitrogen intermediates[84].

We have described various immunological mechanisms that play important roles in protective immunity against parasites. Although specific mention was made of a selected few parasites, similar mechanisms operate for other parasitic infections: for example, $CD8^+$ cells are also involved in protection against *Trypanosoma cruzi*[85]; differential induction of T_h-cell subsets, similar to that in leishmanial infection, is also observed in *Trichinella spiralis* infection[86].

Immune pressure and polymorphism at the antigenic site

Many parasites have developed sophisticated mechanisms to evade host immune response. Essentially there are two ways by which a parasite may survive host immune attack. The first strategy involves suppression of host immunity by the parasite. This has been discussed in the previous section. In the second strategy the parasite employs antigenic variation to neutralize host immunity.

T-cell epitopes are known to vary in different strains of *Plasmodium*. Analysis of sequences of three different CS proteins revealed that the immunodominant sites for recognition by human T cells correspond precisely to the polymorphic segments of the molecule. There are nine amino-acid changes, due to nonsilent mutations at the nucleotide level in the CS gene. The major variation in CS protein in different isolates centres around the C-terminal region, within the T-cell epitope. T cells primed to this peptide from one strain did not recognize the variant C-terminal sequences in the same peptide from other strains[87,88]. Since antibodies against this region block erythrocyte invasion by the parasite, alteration of this sequence in such a way as to alter antigenicity without changing the ability to interact with the erythrocytes can promote survival of the parasite[89]. Antigenic diversity has also been observed with respect to a number of other antigens associated with *Plasmodium*. Variant clones may be responsible for successive peaks of parasitaemia[90,91].

Antigenic variation has also been observed in other parasites, one of the best studied examples being the variable surface glycoprotein (VSG) of *Trypanosoma brucei*. Variation in the VSG molecule, more particularly in the N-terminal variable portion (antigenic), allows the parasite to evade the host immune system[92]. Synthesis and expression of altered VSG are the result of immune selection rather than immune pressure: trypanosomes with altered VSG can only survive the immune response as the latter is directed against the epitopes of the prevailing VSG.

Changes in the agretope (MHC molecule-binding region) of the CS of *Plasmodium* due to immune pressure have been suggested by de la Cruz *et al.*[93] on the basis of their observations on dominant T-cell epitopes (amino-acid residues 326–343 and 361–380). Altered genetic restriction was observed when some of the variant sequences were used to activate T cells[93]. Stage-specific antigens that are not subject to immune pressure, e.g. the Pf25 zygote/ookinete antigen, which elicits a poor immune response in the host, did not display wide variation/polymorphism[54]. The cysteine-rich cell-surface molecule of *Giardia* also displays marked variation[94,95]. It is not known if these changes are due to immune pressure or immune selection. In many parasites different stages of the life cycle can be defined by changes in the cell-surface carbohydrates[96,97]. Altered surface carbohydrate structures have also been associated with avirulent strains, compared with virulent strains[98,99].

Polymorphism in surface carbohydrate could potentially alter the nature of host–parasite interactions. In *Leishmania*, the surface carbohydrates vary among different strains and also different stages in the life cycle of the parasite. Mechanisms controlling such changes are far from clear.

Genetic basis of resistance/susceptibility to parasites

The majority of parasitic infections display a broad spectrum of immunopathological responses. Apart from variation in the parasite, genetic variation in the host may play a vital role in determining the different disease profiles observed clinically. Resistance and susceptibility to infection may be governed by a number of factors.

Failure of parasite to gain access to host tissue

Expulsion of parasite egg larvae from the host has been

shown to be genetically determined in many human gastrointestinal helminth infections. Resistance is inherited as a dominant character and more than one gene is involved[100].

Absence of appropriate receptors for attachment and entry of parasite into target cells

In certain parts of West Africa, individuals of the Duffy-negative blood group show much lower incidence of *Plasmodium vivax* infection as the parasite cannot enter the erythrocytes because of the absence of suitable receptors on erythrocytes of Duffy-negative individuals[101].

Failure of parasite to survive within host cells due to presence of antiparasitic factors

Complement components are toxic to many parasites. Parasites have a thick extracellular coat of complex carbohydrates or glycoproteins along with other surface components that inactivate the complement pathway to protect them against lysis by complement, e.g. VSG of trypanosomes[102]. Thus individuals with defective complement activation would have altered course of infection. The third component of complement (C3) is responsible for uptake and intracellular survival of *Leishmania major* within macrophages[103,104]. The infective stage of this organism has an altered mechanism of activation of C3 and further processing of the product[105].

Failure of parasite to undergo further development within the host due to absence of host signals or nutritional factors

Cyst formation in mice following *Toxoplasma gondii* infection is controlled by a set of at least five genes, one of which is linked to the *H-2* complex[106].

Destruction of parasite after entry into tissues

Granulocytes and phagocytes, with or without the help of immunological mediators, are capable of destroying many parasites. Some strategies against these cells that parasites have developed include inhibition of phagocytosis and further steps in killing within these cells. Genetic defects in the ability of these cells to get activated or to generate reactive oxygen intermediates enhance susceptibility to parasite infection. In experimental murine models an *Ish* gene segregates into dominant resistant and recessive susceptible alleles[106]. The gene maps to chromosome 1 in mouse and may be identical to *Ity* and *Bcg* loci, which control early infection by *Salmonella typhimurium* and *Mycobacterium tuberculosis* respectively. The gene regulates some form of T-cell-independent macrophage activation process. Restriction-fragment-length-polymorphism (RFLP) analysis of this region indicates that the *Ish* gene is located in a segment of the chromosome that contains a large number of cytoskeletal-protein genes, including collagen and myosin[107]. All attempts to clone this gene have been unsuccessful so far. The *Scl1* gene regulates susceptibility to *Leishmania major* infection. The defect is thought to be again in macrophages[106].

Failure to generate appropriate immune response

Generation of immune response leading to protective immunity may be dependent upon host factors that regulate immune response. As discussed earlier, MHC molecules determine the level of both antibody and T-cell response. Antibody response against CS proteins is restricted to only a few strains of inbred mice. Such limited class-II-molecule-restricted response has also been observed with antigens derived from other parasites[109]. Even suppressive response mediated by parasites was found to be genetically restricted[110]. Attempts are being made to make synthetic molecules containing T-cell epitopes that will elicit high response in a number of different class-II molecule-bearing strains, along with protective B-cell epitopes, so that they could be used to vaccinate the outbred human population[111].

Epitopes, MHC and immune response: Molecular basis for vaccine development

Structure of MHC class-I and class-II molecules

The genetic loci and the gene products encoded in the major histocompatibility complex (MHC) have been characterized in several species, more extensively in man (HLA) and mouse (H-2). Two different classes of gene products of MHC directly participate in immune response: class-I molecules are highly polymorphic glycosylated polypeptide chains of about 45 kDa, associated on the cell surface with a non-polymorphic, non-glycosylated, non-MHC-coded polypeptide chain, the 12 kDa β_2-microglobulin; class-II molecules consist of two polymorphic glycosylated polypeptide chains of 27–31 kDa, and are present on the cell surface as a heterodimer. Both class-I and class-II molecules are anchored on the cell surface through domains that span the lipid bilayer. While class-I molecules are present in almost all cell types, class-II molecules are expressed only in selected cell types[112]. The genes for many class-I and class-II molecules have been cloned and sequenced. Detailed analysis of the primary structure (amino-acid sequence) of the proteins suggests not only the existence of multiple domains but also that the domains are very similar to those of immunoglobulins. Molecular-genetic analyses of the corresponding genes have shown a striking correlation between the exons

and putative polypeptide domains[25]. The crystal structure of a class-I protein at 3–5-Å resolution revealed that the variable domains contribute one helix each. Eight antiparallel β-sheets lie underneath the helices. The residues lining the groove formed by the helices and the β-sheets, as well as the top part of the helices, are part of the most polymorphic region of class-I sequences. Therefore it has been suggested that the helical groove may be the peptide-binding domain of class-I MHC molecules[113].

The polymorphic regions of the α and β polypeptide chains of the class-II MHC molecules reside in the N-terminal domains. A model for class-II molecules, based on common features of class-I and class-II-molecules, has been proposed, which resembles very closely that of HLA-A2 (a class-I protein), with two helices supported by eight β-pleated sheets[112].

Prediction of antigenic epitopes

The polymorphism of MHC proteins probably influences both antigen presentation and antigen recognition by TCR. Polymorphic residues in and around peptide-binding sites of MHC molecules may influence selection of certain fragments from the whole antigen molecule and subsequent interaction with TCR. Interaction of MHC with antigen/peptide has been studied by both direct-binding[114–117] and peptide-competition studies[118,119]. Buus *et al.*[116,117] showed, using a number of different peptides, that each peptide bound its functional restriction element and that, in most cases, binding to the cognate MHC molecules was stronger than to other MHC molecules studied[120]. On the other hand there is enough evidence to suggest that mere binding to MHC is not sufficient for eliciting T-cell response. One of the major advantages of *in vitro* studies on peptide selection in specific MHC-molecule contexts is the ability to identify peptides with desired qualities, one of them being that of eliciting high response in different MHC contexts (haplotypes) *in vivo*.

Peptides could be selected that bind different MHC class-II molecules. Sinigaglia *et al.*[121] demonstrated that the *Plasmodium* CS-antigen peptide 378–398 can bind to most human HLA–DR molecules and induce proliferation of T cells that would recognize native CS. Such a peptide could be a good candidate for an antimalarial vaccine[122].

As pointed out above, binding of a peptide fragment to an MHC molecule is not sufficient to induce T cells to proliferate. It may be that a peptide may not be immunogenic by itself but may be capable of competing with other immunogenic peptides (e.g. those derived from parasites) for binding to the same MHC molecule. This may lead to inhibition of T-cell response against the immunogenic peptide. Such peptides could be useful in controlling unwanted immune response that may lead to pathophysiological damage, as in the case of some parasitic diseases[118].

Many attempts have been made to predict T-cell epitopes based on primary-sequence data in spite of the fact that the conformation of processed antigen may be very different from that of the native protein[123].

Epitope displays helical conformation. A number of presented peptides do display helical conformation. The 28-amino-acid fragment from the 70-kDa merozoite protein of *Plasmodium falciparum* has helical conformation[124]. Computer modelling suggests that helical conformation could allow peptides to interact with MHC molecules. On the other hand irregular conformation could still yield a good fit in the helical groove of the MHC molecule. Not all presented peptides have helical conformation. In many instances there is no correlation between a peptide's propensity to form a helix and T-cell proliferation.

Epitope displays amphipathic character. Many parasite-antigen epitopes have been described that display amphipathic conformation, which can be predicted using a computer program. CS-antigen peptide 326–342 (Th2R) was found to have a high amphipathic score and stimulated T cells of $H\text{-}2^k$ haplotype[49]. However, the three-dimensional structure of HLA-A2 (a class-I protein), as revealed by X-ray crystallography, did not show any specific requirement for amphipathicity. In fact many presented peptides do not have amphipathic character[123].

Consensus sequences based on data banks. Due to the absence of large data banks no consensus sequence has yet been demonstrated in the case of T-cell epitopes. The same is true for MHC-specific consensus sequences. However, many epitopes have a four- or five-residue peptide sequence in which a charged residue or glycine is followed by two or three hydrophobic residues and ends with a polar residue[125]. Such a sequence could also form an amphipathic helix.

Search for T-cell epitopes using overlapping synthetic peptides to challenge lymphocytes from naive donors has tremendous potential to identify fragments that have a wide HLA requirement. Such an approach led to the identification of a peptide from the CS antigen that was found to associate with at least seven different HLA-DR proteins[121]. There is one major disadvantage in this approach: epitopes that react with T cells from naive donors may be different from epitopes that react with immune cells. However, in some experiments CS-protein epitopes were recognized by both immune and naive T cells[122].

There have been a number of attempts to predict antigenic sites that would be recognized by antibodies from primary- or secondary-structure data. Since most

antibodies are thought to be directed against conformational determinants, it is reasonable to assume that antigenicity is a surface property. Several other structural features, namely hydrophobicity[126], β–turns[127], segmental mobility[128], etc., have also been considered. Most of these features may reflect accessibility of the residues to the outer environment. Antibodies generated against the predicted peptides must react with the native protein. These predictive methods have been only partially successful[123].

Concluding remarks

Precise immunological mechanisms necessary and/or sufficient for resistance to pathogens remain largely unknown for most pathogens, except, perhaps, for a few viruses. In spite of the tremendous development in the understanding of basic immunological processes we are ignorant about the role of different immunological reactions in parasite growth, development and eventual adaptation to human and animal hosts. It seems clear that, in many parasitic infections, antibodies do not play any role in protection. The evidence in support of this conclusion comes from experiments carried out in both humans and animals where either no significant antibody titres could be detected in immune individuals or high antibody levels were found in patients suffering from active disease. It is likely that absence of correlation may relate to inability to identify, as yet, parasite antigens that directly participate in the disease process. On the other hand T cells seem to be involved in protective mechanisms. Killing of parasites by macrophages activated by IFN-γ secreted by T cells is thought to be one of the main protective mechanisms. In many instances CTL may kill target cells containing intracellular parasites and expressing parasite antigens.

Attempts to develop vaccines for parasitic diseases have not been successful in spite of early promise offered by the work on CS protein, a malarial antigen. Limited vaccine trials in man suggest a very complex range of immune response involving a number of factors, including host genetic differences and antigenic variation in parasites.

Molecular approaches to vaccine development, involving methods to search for T- and B-cell epitopes, have been used extensively. Results suggest that no single epitope may be able to elicit adequate immune response that may lead to protection. Large molecules containing multiple epitopes (MAP, multiple-antigen peptide) spanning fragments that associate with different MHC proteins, along with suitable B-cell epitopes, may be more effective in eliciting protective immunity. On the other hand use of specific vectors, e.g. *Salmonella*, vaccinia virus etc., may help in eliciting specific immunity by stimulating only one of the arms of the immune system, e.g. T-cell response in absence of B-cell response. In many instances cross-reacting T-cell response could be generated against evolutionarily conserved stress proteins that may have broader implications in host immunity. Some of these proteins have already been characterized partially.

There has been encouraging progress in defining candidate protective antigens in many parasites. There is a need to identify more such molecules. Many of these antigens undergo rapid variation, which allows the parasite to evade immune pressure. The molecular mechanisms that regulate such variation are not yet clear in most cases and must be studied to design strategies to counter them. Better understanding of the pathogenesis of infection and development of strategies to modify immune response to kill the parasite may lead to reduction of the rate of parasitic infection, a goal that we may be able to achieve in the next decade.

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DNA profiling and its applications

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Every individual in the world can be identified at the molecular level on the basis of an extremely high level of polymorphism in the sequence of his or her DNA, which he or she inherits from his or her biological parents and is identical in every cell of the body. DNA fingerprinting, as this technique of identification is called, can confirm with certainty the parentage of an individual. Its applications are, therefore, in the establishment of paternity for personal reasons, in affiliation, wardship or divorce proceedings, in testacy disputes, and in provision to immigration authorities of clear evidence of a family relationship. The test has major applications in the field of forensic science, where samples left at the scene of a crime may be analysed against those from a person suspected of committing that crime; in the veterinary field, where pedigree confirmation may be required; in the medical world, for the diagnosis of genetic diseases; and in agriculture, for identification of seed stocks and germplasm. DNA probes derived from a DNA sequence first isolated from an Indian snake are useful in forensic investigation by DNA fingerprinting.

Crime is as old as human civilization and so is man's ingenuity in crime investigation. The accumulation of special knowledge and inventiveness has been a hallmark of our approach in solving all types of problems. Application of developments in the field of science and medicine in solving legal problems is a good example. It is fascinating how the idea of fingerprints was first introduced in India in 1860 as a proof of identity for the payment of military pensions to illiterate ex-soldiers by a British administrator, William Herschel, to stop widespread swindling. The extensive study and classification of fingerprints, necessary as prelude to their full use in crime investigation, was carried out by the English biologist Francis Galton, whose classic book *Fingerprints* was published in 1892. Based on this, the first murder case was solved in 1892 in Argentina. In Britain, the full adoption of fingerprints had to wait a few more years until the introduction of a new system of classification by Edward Henry, which is even now used all over the world. The estimated probability of fingerprint match in the human population is one in 10^{10}.

It has long been the ambition of the forensic scientist to be able to identify the origin of blood and body-fluid stains found at the scene of a crime with the same degree of certainty as fingerprints. Development of electrophoretic and immunologic techniques to type material in respect of polymorphic proteins and cellular antigens helped in fulfilling this ambition to a certain extent. However, while discrimination using genetic markers is conclusive in exclusion, the chance of inclusion or positive identification using these markers does not exceed 99.7% for a total of 20 polymorphic systems used together (HLA, red cell antigen, serum proteins, red cell enzymes)[1]. Besides, blood evidence from the scene of crime usually enters the laboratory in the form of dried stains, and the drying, ageing and contamination associated with these stains limits the number of markers for which a sample can be typed. There has been, therefore, clearly a need to have another type of marker that not only survives better than proteins but also possesses a larger number of distinguishable alleles. This need was fulfilled by the