Part I

Novel Vaccination Strategies. Edited by Stefan H. E. Kaufmann

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Challenges for the Vaccine Developer, including Correlates of Protection

G. J. V. Nossal

1.1
Introduction

For four reasons, a book on novel vaccination strategies is particularly timely. First, the stunning advances in basic biology, and particularly in genomics, have opened up new vistas in vaccinology and have introduced a much more solid underpinning of science into a field characterized by a fair degree of empiricism. Second, the devastating impact of the HIV/AIDS pandemic has alerted the world to the imperative need for a vaccine to contain it and, as a side effect, has permitted greater publicity for other major communicable disease killers, such as tuberculosis and malaria. Third, humanitarian issues, such as immunizing all the world's children and not only the children of the rich, have taken on a new urgency after September 11, 2001. As Varmus has argued, 'to a very great, if not measurable extent, terrorism is a manifestation of anger and resentment about the world's inequalities' [1]. Fourth, the quite extraordinary generosity of the Bill and Melinda Gates Foundation has permitted highly significant new sums of money to flow into vaccine research and, through the Global Alliance for Vaccines and Immunization, has raised the prospect that new and improved vaccines might promptly reach those in greatest need of them [2].

That being said, the would-be inventor of new or improved vaccines still faces formidable challenges. The expensive applied research, development work, and clinical trials that are required following the academic research phase are usually the province of industry, which has an understandable requirement to be profitable. Where the vaccine is one chiefly of interest to developing countries, novel strategies of funding and/or execution may be necessary. For example, the Gates Foundation-funded African meningitis program seeking to combat epidemics caused by *Neisseria meningitidis* has engaged a vaccine manufacturer in India, with its much lower labor costs, to develop a carbohydrate–protein conjugate vaccine, with the help of two manufacturers in industrialized countries with some of the raw materials and of technology transfer. Another very real challenge is the fact that most existing licensed vaccines have been developed using antibody levels generated as the key guide. Now vaccines are required where T cell immunity is essential. The correlation between measured T

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cell reactivities and durable protection is frequently much more dubious. Moreover, unfortunately, animal models, particularly rodent ones, have been poor predictors of clinical efficacy. It is therefore important to explore all the mechanisms of protection that the immune system provides and to gradually build up a combinatorial image of what elements contribute to a vaccine that really works. Of course, all the correlative arguments in the world do not bypass the need for phased clinical trials. Part of the challenge will be to learn the correlates of protection from such trial results.

1.2 Mechanisms of Protection within the Immune System

The mechanisms of protection are conventionally divided into innate or primitive and adaptive or acquired, but as later chapters show, these systems are far from unrelated. Indeed, it is valuable to think of bodily defense as a complex, highly integrated series of interacting cellular and molecular processes, some depending on evolutionarily primitive recognition processes and others being much more specific and highly evolved. Moreover, because the most recent system, that which depends on the somatic generation of B and T cell repertoires, evolved in the presence of earlier systems, it is natural that functionally new processes should be 'grafted onto' what went before. Thus it comes as no surprise that antibodies serve their function with a high dependence on the complement system or on phagocytosis. So long as this is recognized, it is legitimate to set out the key elements of the innate and the adaptive systems.

The innate immune system is evolutionarily as old as multicellular life itself, namely about two thousand million years, but its central molecular features have only recently been elucidated [3]. Table 1.1 summarizes its key elements. Probably the most important newer finding is that of the Toll-like receptors for pathogen products of various sorts. These evolutionarily old receptors form a link between the innate immune system and the adaptive system, as is fully discussed in another chapter 2.

Table 1.2 deals with the more familiar mammalian adaptive immune system, which consists of a multiplicity of components. So the question is 'Which element is the most important in protecting the host against infection?' because that is what the vaccine developer should monitor as research on a new vaccine progresses. The matter is not simple, because various elements collaborate in vanquishing infections. Physicians have known for nearly a century that the crisis in lobar pneumonia that precedes recovery signifies a sufficiently high concentration of opsonic antibody to allow lung macrophages to engulf the invading bacteria. Without either the antibody or the macrophage, the patient would die. Yet it is clearly impractical for the researcher to seek to correlate every element of Table 1.2 with the degree of protection afforded by a vaccine. A further complexity is the fact that what leads to *recovery* from infection may not be identical to what is required to *prevent* infection or reinfection. In influenza, for example, cytotoxic T cells are probably the most important element limiting viral spread and thus speeding recovery, but antibodies in respiratory tract mucosal fluid are probably key to preventing an attack. Given these influences, it is not surprising that correlates of protection is a field that has not progressed very far.

Tab. 1.1 Some key elements of the innate defense system.

- Cilia
- Enzymes in mucous secretions, e.g., lysozyme
- Repair mechanisms of damaged anatomical barriers, e.g. the clotting cascade or growth factor and chemokine release
- Defensins
- The complement cascade
- Non-immunoglobulin opsonins, e.g., collectins such as mannose-binding protein or C-reactive protein, lectins, fibronectin, etc.
- Recognition receptors on dendritic cells, macrophages, NK cells, and mast cells including Toll-like receptors, scavenger receptors, or integrin
- Phagocytosis by polymorphonuclear leukocytes, monocytes, or macrophages
- Cytokines, including interferons α, β, γ and tumor necrosis factor

Tab. 1.2 Some key elements of the adaptive immune system.

- B lymphocytes, their precursors and progeny, and their products – 8 different classes of antibody
- T lymphocytes with α/β receptors, their precursors and progeny, their lymphokine products, and specifically $CD4^+$ regulatory (suppressor) T cells; $CD8^+$ cytotoxic and cytokine secretory cells; and CD1-specific $CD4^+$ or double negative NK-1 T cells
- T lymphocytes with γ/δ receptors, their various subsets, and their lymphokine products
- Other atypical T cells
- Fc receptors of various types on monocytes, macrophages, immature dendritic cells, B cells, polymorphonuclear leukocytes, NK cells, mast cells, and platelets, as well as soluble Fc receptors

The most convenient way of discussing present understanding is to deal separately with viral, bacterial, and parasitic pathogens.

1.3 Protection against Viruses

Virus infections can be relatively localized, e.g., to the respiratory or alimentary tracts (a more challenging situation for the induction of protective immunity) or they may spread systemically first via the lymph and then through the blood, where opportunities for engaging the major sites of immune induction are plentiful. Virus infections may be acute and followed either by death or by complete viral elimination; they may set up a latent infection, going underground in particular cells, only to reemerge sporadically and unpredictably, as with herpes viruses; or they can set up a chronic infection as in hepatitis B or C. Knowing the respective life histories of the viruses is important to devising appropriate vaccine research strategies.

The innate immune system offers a substantial defense against viruses. In particular, the type 1 interferons, α and β, damp viral replication and thus limit their spread. NK cells become activated and, through cytotoxic activity, kill virus-infected cells. The

innate immune system also provides a variety of other potent cytokines. Macrophages have antiviral functions but can also harbor viruses and aid their spread.

Adaptive immune responses come in somewhat later and usually involve both antibodies and T cells. The general rule is that antibody, with its capacity to recognize structures on the surface of a virus, plays the main role in neutralizing free virus, whereas T cells, recognizing viral peptides processed intracellularly and presented on cell surfaces, have virus-infected cells as their target. In both pathways, it is important to delineate which viral antigens are the most important. As far as antibody formation is concerned, the key molecules are those that the virus uses to gain entry into the host cell. Covering these up with antibody prevents the initiation of infection, and such antibodies are known as neutralizing antibodies. Surface glycoproteins or outer capsid proteins are the most critical viral antigens that induce neutralizing antibody. Viruses having a lipid envelope need not only to attach but also to fuse with the host cell membrane. Often, a separate fusion protein exists, which is also a neutralization target. Newly formed viruses need to be released from the infected cell. With influenza virus, for example, release is achieved via a specific molecule, neuraminidase. Although not as important as antibodies to hemagglutinin, which binds the influenza virus to the cell, antineuraminidase antibodies also participate in protection. Ancillary to these highly specific antibodies are many other antibodies that recognize the virus surface and act in an opsonic manner. Finally, antibodies to any surface structure on a virus-infected cell can lead to that cell being killed by antibody-dependent cellular cytotoxicity or by complement-dependent lysis, aiding control of infection. At the same time, many antibodies are formed to internal virus components that are irrelevant to protection.

In some instances, the process of viral entry is more complex, requiring two receptors. The classical case is HIV, where the gp 120 envelope protein binds to CD4 and, subsequent to this binding, an allosteric change allows the binding of another part of gp 120 to a coreceptor, namely CCR5 or CXCR4, which are members of the chemokine receptor family. It is believed that then a further conformational change leads to insertion of a hydrophobic amino-terminal fusion peptide of gp 41 into the target cell membrane. Cross-linking via gp 41 finally results in membrane fusion and viral entry. The newly and briefly exposed antigenic determinants of gp 120, which bind to the coreceptor, appear to be more conserved than the CD4-binding epitopes and may constitute attractive vaccine candidates [4].

The T cell side of antiviral defense is entirely different. Here, any viral protein, be it surface-located, internal, or indeed nonstructural, may be presented on the surface of an infected cell or of an antigen-presenting cell that has engulfed an infected cell or portions of it. Although classically, peptides from a cell in which virus is growing are presented by MHC class I molecules, and peptides derived by endo- or phagocytosis are presented by class II molecules, there are now numerous examples of cross-presentation. Conventionally, we think of $CD8^+$ cytotoxic T cells recognizing peptides presented by class I as the chief protectors for vanquishing a virus infection. However, the $CD4^+$ T cells recognizing peptides presented by class II are also hugely important. They act as helpers for the production of high-affinity antibody. They also provide help for the activation of $CD8^+$ T cells, produce cytokines with antiviral activity, and recruit phagocytic cells.

In some ways, therefore, the vaccine researcher seeking to develop a vaccine for a disease in which T cell immunity is paramount for protection suffers from an embarrassment of riches. In looking to see if a putative vaccine has engendered a T cell response, it is truly difficult to choose among the different viral proteins, so often 'cocktails' are used, rendering the test less precise than it might have been. Furthermore, it is difficult to know whether to go with whole viral proteins or with known T cell epitopes from a given protein. If the latter, the investigator must face the polymorphism of the human population for both class I and class II HLA molecules and either run the test with a variety of T cell peptides or choose to learn from only a given MHC haplotype.

In infections that relapse (like herpes) or that persist (like HIV), the vaccine developer has to do better than nature, which is of course quite a challenge. Success may depend on a T cell response more precise and/or more intense than that accompanying the infection.

For cytopathic viruses that kill or heal, leaving protective immunity, the correlate of protection is straightforward, being neutralizing antibody. Often, a combination of animal experimentation and clinical seroepidemiology has given good information on the actual levels or titers of antibody required for protection. These then guide choice of antigen dose in the vaccine and the number of injections required. To a degree, they also dictate how often booster doses are required, as in smallpox or yellow fever, although in practice protection may well last longer than threshold serum antibody levels, presumably because of very rapid memory responses should the virus gain reentry. Memory responses obviously take a few days to become manifest, since lymphocyte multiplication and differentiation are required. They are therefore most effective in systemic infections where the pathogen takes a few days to move from the point of entry via the skin or mucus membrane into the lymph, tissues, and blood. They are less effective in purely localized mucosal infections.

Things are a little more complex in a disease like influenza, which starts in respiratory epithelium but becomes more serious as it spreads more deeply. Further, in this disease the key antigens and particularly the hemagglutinin exhibit very high mutation rates. Here minor changes due to an accumulation of point mutations, and known as antigenic drift, are dealt with by a clever, arduous global process, where vaccine manufacturers, guided by World Health Organization collaborating centers, include in the vaccine the most recent circulating variants, with susceptible people being counseled to have yearly boosters. On the other hand, when genetic reassortment occurs between different viral strains, frequently a wild human virus and an influenza virus of an animal such as a pig or a chicken, preexisting immunity is frequently lacking and a pandemic can sweep the globe. These are known as shift variants. It appears that major pandemics have occurred about three times a century over the past three centuries or so.

Should we be looking to correlate protection with IgA present in respiratory mucus or intestinal fluids for respiratory or intestinal infections, respectively? From a practical point of view this has not proven to be necessary, because measuring serum (mainly IgG) antibody seems to do the job. Important as IgA is, we must remember that significant quantities of IgG enter these fluids as a transudate.

Should we be measuring virus-specific T cell levels? For the 'kill or cure' diseases, the undoubtedly helpful T cell responses engendered by vaccines are really a bonus, and it has not proven necessary to document their numbers or persistence. As we shall see below, the situation is quite different for diseases such as HIV/AIDS or hepatitis B or C.

There is a major difference between viruses that are mainly confined to body surfaces and those that become systemically disseminated. The former induce mainly mucosal immunity, which is much shorter-lived than systemic immunity. The same is true for mucosal vaccines, which tend to cause a shorter duration of protection. Still, in 'real life', significant serum antibody is also present and acts as a guide for the vaccine developer.

1.4 HIV/AIDS as an Example of a Persisting Virus

Many of the dilemmas facing the researcher seeking correlates of protection in a chronic virus infection are illustrated by the HIV/AIDS situation. Great early disappointment followed the realization that monomeric gp 120 protein given with adjuvant to human volunteers elicited antibodies that could neutralize laboratory-passaged HIV strains but not fresh primary isolates from patients [5]. Given the prominence of antibodies to the V3 loop and the extreme mutability of the envelope protein in this region, this really should not have been too surprising. But it is far too early to suggest that antibodies will never act protectively. Appropriate antibodies may, in times represent a useful correlate marker. We now possess a much better picture of the events that occur when HIV docks with the $CD4^+$ cell and finally enters it [6]. When gp 120 engages CD4, a stable conformational change occurs which exposes a binding site for a coreceptor, usually CCR5 or CXCR4. This site is highly conserved, as also is the actual docking site for CD4 within the gp 120 molecule. Both sites are in recessed pockets on the inner core of the gp 120 molecule. After both CD4 and the coreceptor have engaged, the noncovalently linked gp 41 molecule changes conformation to reveal a hydrophobic fusion domain allowing viral entry into the host cell. Moreover, the gp 120–gp 41 dimers exist on the virus surface in triplets constituting a viral spike. Additional epitopes may be generated as a result of this trimerization, and what one wants is high-avidity binding to the trimer. It is not easy to elicit antibodies to these conserved elements, but some human subjects with HIV do manage to generate antibodies capable of neutralizing a broad diversity of HIV isolates. Perhaps the most informative approach has been the study of human monoclonal antibodies with this capacity [7]. The structure of one of these, b12, has been resolved to 2.7 Å and possesses a finger-like projection, which is the third hypervariable region of the heavy chain, which pokes into the recessed CD4 binding site of gp 120. Another (2G12), surprisingly, is directed against oligomannose epitopes clustered on one face of gp 120, raising the possibility of a carbohydrate vaccine. Such examples, and the clear proof that passive antibodies can protect against chimeric simian–human immunodeficiency virus (SHIV) challenge, offer the hope

that eventually immunogens will be generated that are capable of eliciting antibodies with the right characteristics.

This being said, overwhelmingly the most effort is being directed at vaccine approaches capable of inducing $CD8^+$ cytotoxic T cell immunity. These are described in detail in another chapter 5. What is the evidence that HIV-specific cytotoxic T cells will be protective? The accumulation of evidence is impressive [4]. It began with the demonstration that human $CD8^+$ T cells can suppress virus replication in autologous $CD4^+$ T cells in vivo [8], which was soon followed by the observation that the appearance of large numbers of specific cytotoxic cells during primary infection with HIV correlated in time with a substantial fall in virus load. In the SHIV model, vaccines that elicit high CTL levels work, at least to some extent. In the opposite direction, when CD8 T cells are depleted, monkeys develop very high levels of viraemia. Further, clinical nonprogressors with long-maintained low viral loads have high CTL levels [9], as do those heavily HIV-exposed sex workers who remain uninfected [10]. In the absence of absolute proof, most investigators would rank specific cytotoxic T cells as the best surrogate marker for efficacy.

At the same time, $CD4^+$ cells should not be forgotten [11]. Although the exact mechanism is unknown, $CD4^+$ cells are helpers for $CD8^+$ T cell development. Of course, they are required for high-affinity antibody responses as well. Patients with low viraemia tend to have high $CD4^+$ T cell proliferative responses to HIV antigens. Thus, it is probable that a good vaccine would induce both specific $CD4^+$ and $CD8^+$ responses (Table 1.3).

Tab. 1.3 Possible correlates of protection in HIV/AIDS.

Surrogate marker	*Comments*
Serum antibody	Best against actual conserved docking epitopes of virus; hard to generate. Some value against other epitopes, but mutability of virus is a problem.
Antibody in vaginal or rectal mucus	Hard to measure routinely.
$CD8^+$ cytotoxic T cells	Which antigens and which epitopes to use for precision of measurement? Will tetramer staining prove helpful eventually?
$CD4^+$ helper T cells	Which antigens? Which subtype?
Combination of all the above	Best hope in the long term.

1.5 Protection against Extracellular Bacteria

Strategies of host defense are different for bacteria that live extracellularly, for which antibodies are the chief agents of control, versus bacteria adapted to intracellular per-

sistence, where T cells are key. With the former type, bacteria that remain localized, e. g., *Staphylococcus aureus* causing impetigo or boils, are less susceptible to conquest by antibody than bacteria that invade the body, e. g., *Streptococcus pneumoniae* causing pneumonia, meningitis, or septicaemia.

The exotoxins, which some bacteria manufacture to aid tissue destruction, allowing them to get a foothold in the host, are very important targets of immune attack. Suitably treated either chemically (to create a toxoid) or genetically (to create a non-toxic but antigenically similar analogue), such molecules become very effective vaccines. Typical examples include *Clostridium tetani* and *Corynebacterium diphtheriae*, in which the relevant toxoids are among the most effective vaccines in common use. Other bacteria, such as *Clostridium perfringens* or *Bacillus anthracis* make more than one exotoxin. For some species, such as *Bordetella pertussis*, it is preferable to give a combination acellular vaccine, containing not only toxoided pertussis toxin but also other molecules that contribute to virulence, such as filamentous hemagglutinin, pertactin, or fimbrial proteins. In all these examples, antibody levels give the best correlate of protection. This is not to say that T cells do not play any part in defense, but simply that, in a practical sense, measuring the appropriate antibody is the best guide for the vaccine developer.

Another important group of antigens that evoke protective antibodies are the capsular polysaccharides of the bacterial cell wall. Unfortunately for the vaccine developer, genetic polymorphism in these carbohydrate epitopes, even within a single bacterial species, is common. An extreme example is *Streptococcus pneumoniae*, in which over 90 serologically distinct capsular polysaccharides exist, and over 20 of these serotypes are important in human disease. Furthermore, when these carbohydrate antigens are used as vaccines, they prove to be suboptimal in several ways. Infants under 1.5 years old respond poorly or not at all. Beyond this age, both children and adults mount an antibody response without T cell help, which is generally of low affinity and results in poor B cell memory. Nevertheless, a 23-valent carbohydrate-based vaccine has proven helpful in elderly individuals.

A major breakthrough occurred when researchers realized that this type of vaccine could be much improved by conjugating the carbohydrate moieties to a protein carrier such as diphtheria toxoid or mutated toxin. The protein carrier provides T cell help, allowing earlier responses, affinity maturation, and good memory. The first practical fruits of this approach were vaccines against *Haemophilus influenzae* serogroup B, or Hib [12, 13]. There are six capsular serotypes of *H. influenzae*, a to f, capable of causing human disease; fortunately, serotype b is overwhelmingly the most important. When the Hib conjugate vaccine was widely deployed in industrialized countries, it was surprisingly effective and Hib meningitis was virtually eliminated – partly because nasopharyngeal carriage rates were greatly reduced and 'herd immunity' became manifest. Once again, for the development of this vaccine, antibodies were the obvious correlate of protection.

Exactly the same principles have been applied to a pneumococcal conjugate vaccine, where it has not proven possible to include 23 serotypes, but 7-valent, 9-valent, and 11-valent vaccines have been developed by various manufacturers. The serious pathogen *Neisseria meningitidis* serogroup C has also yielded to this approach. A re-

cent widespread deployment of this vaccine has reduced the incidence of meningococcal C meningitis in the United Kingdom by over 80% and of deaths by >90% [14]. In Sub-Saharan Africa, vicious epidemics of meningococcal A meningitis sweep across the continent every few years. A carbohydrate vaccine is moderately effective in outbreak control, but the World Health Organization in conjunction with the Program for Appropriate Technology In Health (PATH) has an ambitious goal of developing and deploying a meningococcal A conjugate vaccine to be given to everyone under 21, as well as to be included in routine infant immunization.

Another example of interesting research on conjugates is *Salmonella typhi*, for which the carbohydrate Vi antigen delivered parenterally is somewhat protective, but hopes are high that Vi-protein conjugate vaccines will work better.

Much work is being directed at beating the polymorphism problem by seeking antigens that (1) are exposed at the bacterial cell surface; (2) exhibit limited or no polymorphism; (3) are important determinants of virulence; and (4) do not cross-react significantly with 'self' antigens. Genome mining in those instances where the DNA sequence of the relevant bacterium has been determined is playing a major role in this research.

Entirely different approaches to immunization have met with some success in diseases caused by gastrointestinal-tract pathogens. First, there is the possibility of introducing killed whole bacteria or important virulence antigens in conjunction with a mucosal adjuvant. A vaccine based on whole killed *Vibrio cholerae* together with purified B subunit of the cholera toxin (CTB) is licensed in some countries. Experimental oral molecular vaccines work in animal models of *Helicobacter pylori* disease [15]. Second, extensive investigation of live attenuated bacteria, also given orally, shows some promise. At the moment, the only licensed version of such a vaccine is the Ty21a strain of *Salmonella typhi*. Serum antibodies do not represent a particularly reliable correlate of protection in these diseases, and much of the developmental research has relied on protection of volunteers against challenge administration of the relevant pathogen. Unfortunately, this is not always a good predictor of the results of phase III clinical trials.

1.6
Protection against Intracellular Bacteria

Intracellular bacteria have evolved mechanisms to foil the usually very efficient phagosome–lysosome system of macrophages and other cells and have learnt to live most of their lives either inside a phagocytic vacuole or, having learnt to escape the phagosome, within the cytoplasm of the host cell. In these hidden locations, they are protected from serum antibody, but because peptides derived from them are expressed on the surface of the infected cell, infections can be controlled by T cell immunity. It was established over a century ago that immunity to extracellular bacteria can be passively transmitted via serum [16], but it was also soon established that this did not occur with certain infections, e.g., tuberculosis. Nevertheless, solid immunity to intracellular bacteria does exist, and which can be transferred from immune to naïve ani-

mals via lymphocyte cells [17] (which were soon shown to be T cells). Long before the difference between T and B cells was understood, a skin test to establish T cell immunity (delayed-type hypersensitivity) had been developed and widely used. The diseases caused by intracellular bacteria include some of the greatest scourges of humanity, such as tuberculosis, leprosy, and trachoma. Tuberculosis has taken on even greater significance since the HIV/AIDS pandemic, because infections that may have been well controlled flare up when T cell function wanes. The HIV/AIDS pandemic has also put the spotlight on intracellular bacteria such as *Mycobacterium avium*, which are relatively harmless unless T cell immunity fails. In general, infections by intracellular bacteria are chronic, and much of the pathology is associated with the immune response, e.g., granuloma formation. Usually immunity is not sterilizing, and the balance between bacterial persistence and T cell protection is somewhat labile.

Both $CD4^+$ and $CD8^+$ T cells contribute to defense against intracellular bacteria [18], the $CD8^+$ T cell being activated because bacteria escape into the cytosol, or because of antigens that escape, or because of cross-presentation.

Various sorts of unconventional T cells also contribute to defense against intracellular bacteria. Because Stefan Kaufmann is one of the world experts in this field, please refer to his chapter 21 on tuberculosis for a discussion of this subject.

Will delayed-type hypersensitivity skin tests or in-vitro measurements of T cell function be helpful to the vaccine developer in the search for, e.g., a more satisfactory vaccine against tuberculosis? Certainly, but they are not perfect correlates of protection. A person may yield a florid, highly positive Mantoux test while experiencing a flare-up and wide dissemination of tuberculosis. A negative test in a tuberculosis sufferer is in general a sign of poor prognosis (especially before the antibiotic era) but need not always be so. So the investigator also depends on the inherent plausibility of the antigen(s) chosen and on its efficacy in the most credible animal model available. Because of the chronic nature of the infections concerned, clinical testing of vaccines emerging from basic research is particularly arduous. However, adjuvant formulations and vaccination strategies (e.g., 'prime–boost' protocols) capable of eliciting strong $CD4^+$ Th1 and $CD8^+$ cytotoxic T cell responses are now available and should herald success in the long term.

1.7
Protection against Parasites

It is difficult to be dogmatic about correlates of protection in parasitic diseases, for the simple reason that there is no single licensed vaccine against any human parasitic disease. To this must be added the fact that most parasitic diseases do not themselves lead to solid immunity, *Leishmania major* being an exception when causing tropical sores. Nevertheless, a great deal of work has been done on animal models. I summarize here what is known about three important parasitic diseases: malaria, schistosomiasis, and leishmaniasis.

Malaria is the most prevalent vector-borne disease in the world. It is caused by protozoa of 4 different species of *Plasmodium*, the most fatal of which is *P. falciparum*.

Clearly this organism has developed powerful mechanisms to evade the host immune response, including an incredible degree of antigenic variation. Because of the chapter 22 dealing specifically with malaria, I will only mention that vaccine approaches are being directed at 4 distinct stages in the parasite's life cycle (Table 1.4). The mosquito injects a mobile sporozoite that exhibits a major circumsporozoite antigen. Antibodies to it or portions of it can block invasion of liver cells and be partially protective [19]. When the sporozoite reaches the liver cell, it develops into a shizont containing 10 000 to 30 000 merozoites. As a result, the hepatocyte presents on its surface peptides (T cell epitopes) from a number of preerythrocytic stage proteins. A $CD8^+$ T cell attack on injected liver cells could materially decrease the total number of merozoites formed and thus be partially protective. Here, T cell immunity could correlate with protection. When the liver schizont ruptures, merozoites are released and invade erythrocytes to begin the blood stage of the cycle. A large number of merozoite surface antigens are being investigated as putative vaccine candidates, alone or, more usually, in various combinations. Here, antibody would be the effective agent. Finally, during the erythrocytic cycle, sexual stages known as gametocytes are formed. They exhibit some strong antigens, antibodies against which can inhibit sexual maturation within the mosquito after it takes a blood meal. It could well turn out that an eventual definitive malaria vaccine will contain antigens acting against all four stages.

A further, more controversial, possibility is to try to neutralize a toxin, glycophosphatidylinositol, of malarial origin. An oligosaccharide from this glycan has been

Tab. 1.4 Vaccine candidates from various stages of *Plasmodium falciparum*

Stage	***Antigen(s)***	***Desired response***	***Comments***
Sporozoite	The major circumporozoite protein.	High affinity antibody	The most advanced vaccine candidate. Nature of adjuvant has been critical.
Liver cell schizont	Various T cell epitopes, guided in part by elution of peptides from infected cells.	$CD8^+$ cytotoxic cells	Research is most advanced for *P. vivax*. A promising, rather new area.
Merozoite	Proteins prominent on the surface. Proteins from internal organelles needed for invasion of erythrocytes.	High affinity antibody	An intense, competetive field. Many antigens nearing the clinic. Combinations also being tested.
Gametocytes	Antigens from surface.	High affinity antibody	The 'unselfish' vaccine. Does not help recipient directly but, widely used, would impair transmission.
Malarial toxin	GPI and oligosaccharides there from (see text).	Neutralizing antibodies	Would be combined with other antigens.

synthesized, conjugated to a protein carrier, and used as a vaccine. It materially reduces pathology and mortality in a murine model [20]. Again, antibodies would be the correlate of protection here.

Most investigators now hold the view that a final malaria vaccine should induce both T cell and B cell immunity.

Schistosomiasis is the most prevalent human parasitic disease caused by a metazoan parasite. It is caused by five different species of *Schistosoma*, of which the most important are *S. mansoni, S. hematobium*, and *S. japonicum*. The most advanced vaccine candidate is the molecule Sm28GST [21]. Mice immunized with it consistently show reduced worm burden, impaired female worm fecundity, and low egg viability. Clinical trials are now ongoing. Evidence suggests that Th2-type $CD4^+$ T cells correlate with protection, promoting IgE formation via IL-4 and eosinophilia via IL-5, but the matter is not straightforward because the ova of schistosomes evoke a Th2 response that aids granuloma formation and subsequent pathology. Similarly, Th1 responses induced by a radiation-attenuated *S. mansoni* vaccine can be protective in animals, but T cells from severely diseased humans often secrete Th1 cytokines, suggesting an involvement in pathogenesis [22]. The line between protective immunity and immunopathology is thus clearly a fine one.

Immunological interest in leishmaniasis was first aroused by the realization that tropical sores, once they eventually healed, resulted in immunity to reinfection. Interest was increased by the clear-cut finding that, in a murine model, Th1 type $CD4^+$ T cell responses cure lesions, but Th2 type immunity leads to progressive lesions and death [23]. The promastigotes of the parasite live inside macrophages. Here the Th1 cytokines, particularly IFN-γ, appear to be able to activate macrophages to kill the parasites. When the infected macrophage eventually ruptures, it releases amastigotes, which have to find another macrophage to infect. Amastigote antigens can be thought of as similar to merozoite antigens; thus, antibodies could theoretically play a role here. However, the experimental evidence that immunity to leishmaniasis is T cell dependent is so voluminous that I put this notion foward with some reluctance.

1.8 Conclusions

It is perhaps a blessing that the by now quite substantial number of investigators who have been seeking to invent new and improved vaccines have not been deterred by the considerable difficulties that need to be faced after the preclinical research has reached its conclusion. I have covered a good many examples in which the correlates of protection are straightforward – substantial levels of high affinity antibodies to one or more important bacterial or viral antigens. We have seen examples in which strong experimental evidence suggests that what is needed is a substantial T cell attack, Th1 in some instances, $CD8^+$ cytotoxic T cells in others, or perhaps both. This leaves several disease problems for which we simply do not know what the correlates of protection will be.

As already noted, vaccine research has always been a combination of fine basic science and enlightened empiricism. From that viewpoint, putative vaccines will simply have to be taken one by one, readied for clinical evaluation when experimental efficacy is convincing and the disease burden sufficient, and studied in humans, with the field trials probably providing the best correlates of protection. It is a grand challenge, particularly now that the position of the less privileged on our globe has been articulated and addressed. I hope that this necessarily cursory overview chapter has whetted your appetite for the many in-depth contributions in the remainder of this book.

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