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Review

Vaccination against and treatment of tuberculosis, the leishmaniases and AIDS: perspectives from basic immunology and immunity to chronic intracellular infections

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Abstract. The occurrence of infectious disease represents a failure of the immune system, a failure that must be prevented by effective vaccination or remedied by treatment. Vaccination against acute diseases such as smallpox and polio are very effective, due to the rapid and increased immune response of vaccinated individuals upon natural infection. In contrast, effective vaccination against intracellular pathogens that cause chronic diseases, such as the leishmaniases, tuberculosis and AIDS, has not been achieved. Clinical observations suggest cell-mediated, Th1 responses, exclusive of antibody production and the generation of Th2 cells, are optimally protective against these intracellular pathogens. Effective vaccination must ensure the generation of such a protective response. We explore here whether understanding very broad features of the regulation of the immune response can accommodate modern findings on the immunological features of these diseases, and provide a perspective within which strategies for effective vaccination and treatment can be developed.

Key words. Vaccination; treatment; tuberculosis; AIDS; leishmaniases; Th1/Th2 cells; immune class regulation.

Introduction

Why is vaccination effective against some pathogens but not against others?

The success of effective vaccination against such acute diseases as smallpox, polio and tetanus contrasts with our failure to reliably vaccinate against intracellular pathogens that cause chronic disease. These diseases include tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, AIDS, caused by HIV-1 virus, and leishmaniases, caused by protozoan parasites. Tuberculosis is still the most devastating infectious disease worldwide, causing about 3 million deaths a year [1], while AIDS has been projected to displace tuberculosis as the number one infectious disease killer in just a few years. We develop in this review a framework for the design of strategies for vaccination against, and treatment of these and perhaps other chronic diseases caused by slowly growing intracellular pathogens. What we discuss and propose is speculative: there is no consensus on how effective vaccination and treatment can be realised, and the real tests of

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such strategies are field trials, which have yet to be carried out.

The critical starting point must be to identify differences in the immune response between individuals or animals that resist an infection, called healthy contacts, and those who suffer disease, thereby providing clues as to the nature of the immune system failure. The most discussed and generally plausible possibility depends upon the fact that the immune system has different means of fighting foreign invaders, and their efficacy in containing and combating different infections varies [2]. Thus, the resistance of mice immunised against *Clostridium tetani*, the bacterium that produces the tetanus toxin, can be passively transferred to nonimmunised mice by the transfer of immune serum, demonstrating the role of protective antibodies. However, the resistance to *Listeria monocytogenes*, an obligate intracellular bacterium that replicates in macrophages, achieved by infecting with a sublethal dose, cannot be transferred by antibody. This resistance can only be transferred to naive animals by the transfer of immune cells [3]. Such studies provided an operational definition of protective cell-mediated immunity. Protective cell-mediated immunity is often found to correlate with the expression of delayed-type hypersensitivity (DTH): swelling and oedema that peak between 24–48 h after subcutaneous or intradermal injection of the antigens against which the animal or person has been sensitised. The development of this DTH 'skin test' followed naturally from the first recorded observation of DTH by Robert Koch in his attempts to develop therapy for tuberculosis by administering antigenic material derived from *M. tuberculosis* to tuberculosis patients [4]. These attempts followed Koch's discovery that this organism caused tuberculosis. Koch saw a delayed 'reaction' to the injected material, consisting of oedema and swelling.

Why is vaccination effective against some pathogens but not against others? Vaccination against polio virus, smallpox and tetanus ensures a rapid immune response upon natural infection by the pathogen, providing the host with the advantage. However, in the chronic diseases of tuberculosis, AIDS and leishmaniasis, clinical observation often leads to the suggestion that the outcome of infection depends more upon the *type* of immunity generated rather than the *speed* with which it occurs. Individuals who produce a strong, stable and predominantly cell-mediated response against *M. tuberculosis* [5–7], HIV-1 [8, 9] and leishmania parasites [10–12] contain the infections and are relatively symptom free, whereas those individuals that produce only antibody or a mixed cell-mediated/antibody response suffer chronic or progressive disease [2]. Effective vaccination against these intracellular pathogens must thus ensure that the *correct type* of immunity, namely an exclusively protective cell-mediated immunity, is generated upon natural infection. Vaccination that ensures a rapid ineffective humoral response will not be efficacious against such intracellular pathogens. Indeed, it may be detrimental, as discussed below. We shall take the proposition that exclusive cell-mediated immunity is optimally protective for these three pathogens as a fundamental working hypothesis. Rational attempts to achieve the goals of effective vaccination and treatment must rely on what we know of the regulation controlling the class of immunity induced. We consider it worthwhile to explore whether understanding basic immunological regulatory mechanisms can contribute more to achieving effective vaccination and treatment. This will require us to take cognizance of the genetic diversity of the vaccinated population, and other factors that present problems in developing a universally efficacious vaccination protocol.

Immunological considerations

The tendency for exclusiveness between the induction of humoral and cell-mediated immunity

In 1952, Salvin [13] reported that immunisation with very low doses of protein antigens results in an exclusive cell-mediated, DTH response, whereas immunisation with higher doses results first in the appearance of DTH, whose expression declines as antibody is produced. These observations demonstrate the *tendency* for exclusivity between the generation of cell-mediated and antibody responses to a given antigen.

Immunological deviation and differential regulation of distinct classes of immunity

Observations in the mid-1960s to early 1970s provided the foundation for analytical studies of immune class regulation. Animals immunised to produce antibody to an antigen could no longer be induced to express cell-mediated immunity in the form of DTH to this antigen following a regimen of immunisation that induced DTH in naive animals. The immune response had become 'locked' into an antibody or humoral mode. Asherson and Stone [14] christened this phenomenon 'immune deviation'. We call it 'humoral immune deviation' to indicate the nature of the deviation. Parish [15] subsequently demonstrated the reverse phenomenon, in which the establishment of a cell-mediated, DTH response in an animal resulted in an unresponsive state for the induction of antibody. We refer to this state as 'cell-mediated immune deviation'. Collectively, these studies show that the immune response to a particular antigen can become locked into a humoral or a cell-mediated state. It is important to be aware that many if not most immune responses have both cell-mediated and humoral components and, as we shall see below, certain conditions have to be met to establish cell-mediated immune deviation. These immune-deviated states are

antigen specific. A state of humoral immune deviation to an antigen 'A' prevents the induction of DTH to that antigen but not the ability to generate DTH to an unrelated antigen 'B'.

Parish's studies are likely to be relevant to achieving effective vaccination against slowly growing intracellular pathogens that cause chronic disease. Such vaccination is likely to be effective if it guarantees a strong and exclusive cell-mediated response upon natural infection. However, we know of no reports attempting Parish's approach until we decided to do so in the early 1990s [16], as discussed below.

The cellular basis of immune deviation and the definition of Th1 and Th2 cells

What is mechanistically responsible for a state of humoral or cell-mediated immune deviation? Various possibilities can be imagined. For example, immunisation in a manner that produces antibody could result in the disappearance of precursor DTH (pDTH) cells, i.e. result in the absence of lymphocytes that can be activated to multiply and differentiate into DTH-mediating cells, thus explaining why DTH responses can no longer be induced. Parish [15] considered such a possibility. Alternatively, but not necessarily exclusively, humoral immune deviation could be due to the generation of antigen-specific T cells that inhibit the induction of DTH [17]. A study shows this suggestion to be correct [18]. We outline some salient features of the study, because they are critical to our approach to vaccination against and treatment of these diseases.

Mice were immunised to produce antibody and a state of humoral immune deviation specific for horse red blood cells (HRBCs). If this unresponsiveness for the induction of DTH to HRBCs is due to HRBC-specific inhibitory T cells, one should be able to transfer this unresponsiveness to a naive syngeneic mouse by giving it T cells from the humorally immune mice. Transfer of such cells from immune to naive mice did prevent the induction of DTH to HRBCs. Further experiments showed that these cells were Ly1+ or, in more modern terms, CD4+ T cells. Thus, humoral immune deviation is due to the action of T cells able to suppress DTH [19]. We refer to these as TsDTH cells.

A feature of the mechanism by which such TsDTH cells act is fundamental and critical to the conceptual scheme we shall develop. The proposal had been made that TsDTH cells specific for an antigen 'Q' could inhibit the primary DTH response to an antigen 'R', chosen to be very different from 'Q' and so not cross-reactive with it, in the presence of the 'conjugate', Q-R, in which 'Q' and 'R' are physically linked, but not if 'Q' and 'R' are both present but not linked to one another. This proposal was tested by the following experiment. Mice were immunised to the protein antigen haemocyanin (Hm) to produce antibody. The administration to naive mice of T cells from such Hm-immune mice could inhibit the induction of DTH to HRBCs in the presence of the conjugate Hm-HRBC. Inhibition was not observed in the presence of HRBCs alone, or of both HRBCs and Hm in a form not mutually linked, i.e. not in the presence of HRBC and Hm coupled to mouse red blood cells (MRBCs) (see fig. 1). We believe these observations illustrate a general and central phenomenon: T cells specific for one part (Hm) of an antigen (Hm-HRBC) can affect the activation of T cells specific for another part of the antigen (HRBC). We refer to this by saying that TsDTH cells act through recognition of linked antigenic epitopes or, more succinctly, by saying that TsDTH cells act by linked recognition [19].

Further studies in this system showed that the T cells able to mediate DTH were also Ly1+ or CD4+ T cells. In summary, these experiments showed that there are two subsets of CD4+ T cells that differ in two characteristics: the first mediates DTH, whilst the other is generated during the course of an antibody response, does not express DTH, but acts to suppress the induction of DTH through linked recognition [20]. It is natural to identify these two subsets of CD4+ T cells with the Th1 and Th2 clones subsequently defined by the studies of, in particular, Mosmann et al. [21].

Another parallel study was directed at understanding the basis of cell-mediated immune deviation. We examined why mice, expressing DTH, were unable to produce antibody on a challenge that produced antibody in naive mice. Such mice harbour antigen-specific T cells that can inhibit the induction of antibody responses when given to naive mice. We refer to such T cells, able to inhibit or suppress antibody responses, as TsAb cells [21]. These cells were shown to be Ly2+, or CD8+ in more modern terms [19], and represent at least one subset of cells capable of mediating such suppression. There is also the possibility that Th1 cells directly inhibit the generation of Th2 cells. Experiments by others showed that TsAb CD8+ T cells also act by linked recognition [22].

These conclusions can be summarised by the equations:

exclusive DTH → Th1 cells and CD8+ TsAb cells

exclusive Ab → Th2 cells and CD4+ TsDTH cells (that may be Th2 cells)

The biological significance of the differential regulation of distinct classes of immunity

Two simple but significant questions might be raised at this juncture. What is the physiological advantage to the host of having different classes and subclasses of immunity, and why are they differentially regulated [17, 23, 24]? These questions are related. There could be no differential regulation if there were only one class of immunity.

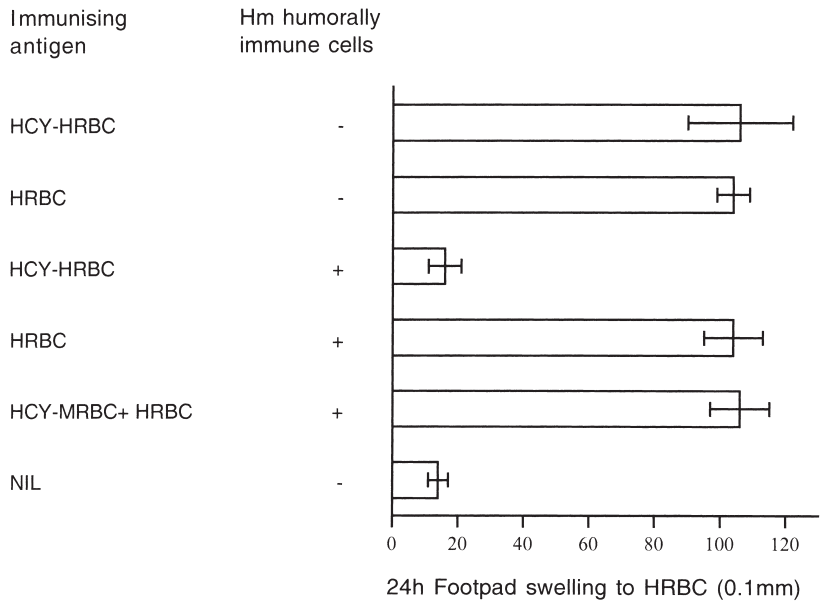

Figure 1. Spleen cells from humorally immune mice suppress the induction of DTH by a mechanism involving linked recognition [based upon ref. 19, with permission]. Mice were injected with either haemocyanin (HCY) coupled to HRBCs (HCY-HRBC) or with HRBCs so that a potent cell-mediated DTH response specific for HRBCs was induced (24-h footpad swelling to HRBC, first two groups). Mice sensitised with the indicated antigens were given spleen cells from mice humorally immune to haemocyanin (Hm) at the time of sensitisation, and the effect on the induction of HRBC-specific DTH was assessed. Spleen cells humorally immune to Hm suppress the induction of DTH to HRBC when HCY is coupled to HRBC (bar 3), but not when HCY is present but coupled to another (mouse) RBC (MRBC, bar 5).

It would initially seem natural for the immune system, on detecting a foreign invader, to discharge all the weapons at its disposal against this invader. However, this is not the case. A decision-making process is embedded within the immune system that results in the differential expression of different classes of immunity under different circumstances. What physiological purpose does such differential expression serve?

Clues come from observations that define the class of immunity effective in containing or attacking different types of target. For example, cell-mediated but not humoral immunity is effective against most cancers [25, 26], against skin or organ grafts from donors minimally different from the host receiving the graft, and against cells infected by relatively slowly growing intracellular parasites [2]. Most of the proteins synthesised in a cell parasitised by these slow-growing pathogens would be host proteins, and so infected cells would only be expected to bear a few foreign sites. Thus the cells of all these entities have predominantly self or self-like antigens on their surface and are 'minimally' foreign. On the other hand, the antigens on the surface of a bacterium are predominantly foreign, and antibody is effective against extracellular bacterial pathogens.

Two generalisations bear on a partial answer to the questions raised above on the physiological significance of distinct classes of immunity and their differential regulation. The first generalisation was made by Pearson and Raffel [27]. They pointed out that some antigens could induce cell-mediated immunity, in the form of DTH [or cytotoxic T lymphocytes (CTLs)], but could not induce the formation of antibody. They identified these antigens as being minimally foreign, either by virtue of being a small molecule, or by virtue of the fact that most of the epitopes of the larger antigenic entity were self epitopes. Examples of the latter type of antigen are cancer cells, or cells of an organ from a donor minimally different from the graft recipient. These are just the kinds of antigenic entities susceptible to cell-mediated but not humoral attack. One might then ask whether any observations suggest why antibody is not effective. Some studies show that antibody-mediated mechanisms of attack are only effective against cells that are more than minimally foreign. For example, one mechanism of antibody-dependent attack is mediated by complement. Complement, recognising and interacting with IgG antibody bound to a cell surface, is activated to create holes in the membrane of the cell, to which the IgG antibody is bound, usually resulting in death of the cell through lysis. Two IgG antibody molecules must be bound close together on the cell surface for complement to attach to them and initiate the lytic mechanism. Studies suggest that several hundred thousand IgG molecules must bind to an RBC for a reasonable probability of an appropriate IgG doublet forming that can lead to complement attachment and initiation of lytic action [28]. However, the number of 'foreign sites' on, for example, cancer cells is likely in the range of a few thousand [29]. Further evidence suggests that other antibody-

dependent mechanisms are only effective if there is a high density of sites on the surface of the cell that are recognised by antibody [30]. Moreover, there is also evidence that CTLs, the lytic cells of cell-mediated immunity, can attack target cells with very few recognisable sites, leading to the lysis of the target cells, whereas IgG-dependent complement-mediated lysis does not occur [31]. We will refer to CTLs as sharp and antibody as blunt weapons. What could be the advantage of this 'bluntness' of antibodies?

Foreign antigens that share structures with a self antigen are known to have the potential for inducing immunity to the self antigen. For example, infection of an individual by group A streptococci can lead to immunity to heart tissue of the individual, due to the fact that some antigens of heart tissue and streptococci are somewhat similar. Such a bacterial infection can result in autoimmunity to heart tissue and hence to rheumatic heart disease [32]. We can imagine situations where the bluntness of antibodies is advantageous when they are generated to self tissue: the antibodies will not damage the self tissue unless the anti-self antibody produced is to very many sites on the self tissue. How could the immune system realise the advantage of having a blunt weapon, as antibody appears to be, thereby minimising damage to self, without compromising its effectiveness to contain foreign invaders?

The above considerations suggest some strategic rules. Suppose a foreign invader has a low density of foreign, recognisable sites. The immune system must generate a cell-mediated response if a response is to be effective. Moreover, since antibody is ineffective, there is no advantage to its production, and there is precedence to suggest that antibody, if induced, could bind to the foreign sites, blocking the access of the effector cells of cell-mediated immunity, such as CTLs, to the target cells [33]. Cell-mediated immunity is apparently needed to contain or eliminate an entity such as a cancer cell, and antibody would be not only ineffective but perhaps detrimental [17, 23, 24]. This perhaps explains the physiological advantage of the inhibition of antibody production during an exclusive cell-mediated response. The generation of any cell-mediated autoimmunity is expected to be highly damaging, a point to which we shall shortly return.

Suppose the foreign invader, such as a bacterium that grows extracellularly, has a high density of foreign sites. Such an invader induces antibody that is effective against it. The advantage of this is clear: any antibody generated to self tissue will be less damaging than would a cell-mediated response to the same tissue. Moreover, if antibody is effective against the foreign entity, a cell-mediated response would not be required to contain it and, if generated, would only have the potential to increase the damage to self tissue. We can thus see a physiological advantage to the association between a strong antibody response and the inhibition of the generation of cell-mediated immunity.

These considerations account for the need for cell-mediated immunity to contain certain types of foreign entities, such as cancer cells and cells infected by slowly growing intracellular parasites, and why antibody is ineffective. The other side of the coin is also well recognised: cell-mediated immunity directed at self tissue is often devastating whereas antibody directed at the same self tissue is not. For example, very interesting studies show that some rodents are healthy despite the presence of T cells with the potential to cause cell-mediated autoimmune disease, such as autoimmune diabetes, that are held in check by Th2-like cells [34].

The decision criterion controlling the Th1/Th2 nature of the response

Many studies have been directed at understanding how antigen interacts with lymphocytes to determine the Th1/Th2 nature of the immune response. The nature of this 'decision criterion' is pertinent to our considerations, because knowledge of this criterion can lead to ways of trying to vaccinate against or treat the infectious diseases we are considering.

Evidence shows there is a precursor T helper (pTh) cell that can be activated in different ways to give rise to effector Th1 and Th2 cells. We start from the premise, for which we believe there is much indirect and direct evidence, that the activation of CD4+ pTh cells in general requires CD4+/CD4+ T cell interactions mediated by the recognition of linked antigenic epitopes. These interactions between specific T cells are envisaged to be mediated by the recognition of antigens presented by antigen presenting cells (APCs), such as B cells or macrophages, by the interacting CD4+ T cells [35, 36].

A valid description of the decision criterion must account for the conditions of immunisation known to determine whether Th1 or Th2 cells are generated. Recall in this context the generalisation of Pearson and Raffel, namely that minimally foreign antigens can only induce cell-mediated, Th1 responses, whereas more foreign antigens can induce antibody, Th2 responses. Moreover, this generalisation seems of central importance to our understanding of how the immune system guarantees an effective response against foreign invaders and minimises the damaging consequences of autoimmunity, as just discussed. This leads to the question: how could the immune system assess the foreignness of an antigen?

The majority of T cells specific for peptides derived from self antigens are eliminated in the thymus. Most CD4+ T cells in the periphery are specific for peptides derived from foreign antigens. In this case, there would be relatively few CD4+ T cells specific for peptides derived from a minimally foreign antigen but many more CD4+ T

cells specific for the greater number of foreign peptides derived from a more foreign antigen. We have also argued that the activation of resting pTh cells requires the antigen-mediated interaction between CD4+ T cells. The threshold hypothesis proposes that few such antigen-mediated interactions of a pTh with other CD4+ T cells gives rise to the generation of Th1 cells, whereas more CD4+ T cell interactions give rise to Th2 cells. Minimally foreign antigens, for which there are correspondingly few CD4+ T cells, will only be able to induce cell-mediated, Th1 responses. More foreign antigens, for which there are many more CD4+ T cells, can induce Th2 cells in the presence of sufficient antigen. However, as these CD4+ T cell/CD4+ T cell interactions are envisaged to be mediated by recognition of antigen presented by an APC, they do not take place at all in the absence of antigen. If the amount of antigen is very low and limiting, these CD4+ T cell/CD4+ T cell interactions will be weak, even for a very foreign antigen, resulting, according to the threshold hypothesis, in a cell-mediated, Th1 response [17, 23, 24]. This is in accord with the findings of Salvin already referred to, and many others, that relatively low doses of very foreign antigens induce exclusive cell-mediated, Th1 responses, while higher doses induce antibody and Th2 cells [13]. Moreover, our recent studies have clearly shown that the number of CD4+ T cells in a mouse and the amount of antigen jointly and interdependently determine the Th1/Th2 nature of a primary immune response, with higher numbers of CD4+ T cells and higher amounts of antigen favouring the generation of Th2 cells [37]. These observations provide critical support for the threshold hypothesis. It is also appropriate to point out here that Th1- and Th2-like cells, once generated, produce cytokines that act directly or in loops to further their own prevalence. Thus interleukin (IL)-4, produced by Th2 cells, stimulates the division of Th2 but not of Th1 cells, whilst interferon (IFN)-γ, produced by Th1 cells, inhibits the division of Th2 but not of Th1 cells [38]. The production of IL-12 by APCs also favours the dominance of Th1 cells [39]. These facts are of central importance to understanding important means of controlling the Th1/Th2 nature of the immune response.

Limitations of the Th1/Th2 view of the immunological universe

We shall assume for the purpose of discussion that there is a spectrum of possible immune states, defined at the extremes by exclusive Th1 and Th2 immunity. This is most likely to be an oversimplification. We pursue discussion within this context, expecting it to reveal in time the limitations of our over-simplified view. In addition, given the number of classes and subclasses of antibody, and their differential regulation, we imagine that there are further insights into the physiological significance of immune class regulation. However, discussion of some of these possibilities would blunt the focus of this article.

Antigen dose determines the Th1/Th2 nature of the response

The threshold hypothesis predicts that antigens, able to induce antibody, will induce exclusive cell-mediated Th1 responses if the dose of the antigen is lowered (and if the antigen does not contain, and is not administered with a substance having, antigen non-specific immuno-modulatory activity, as exists in some adjuvants). This is true for a remarkable array of antigenic entities ranging from proteins [15], xenogeneic red blood cells [40], simian immunodeficiency virus SIV [41], the nematode *Trichuris muris* [42], a murine retrovirus [43], mycobacteria [44–46] and leishmania parasites [16]. This dependence will be central to our discussions of both vaccination and treatment of disease. The conclusion from these observations is depicted in figure 2.

Coherence of the response to different parts of an antigen

Consider a nominal antigen Q that is processed into peptides q1, q2, q3, ... qn that can bind to host class II major histocompatibility complex (MHC) molecules of APCs

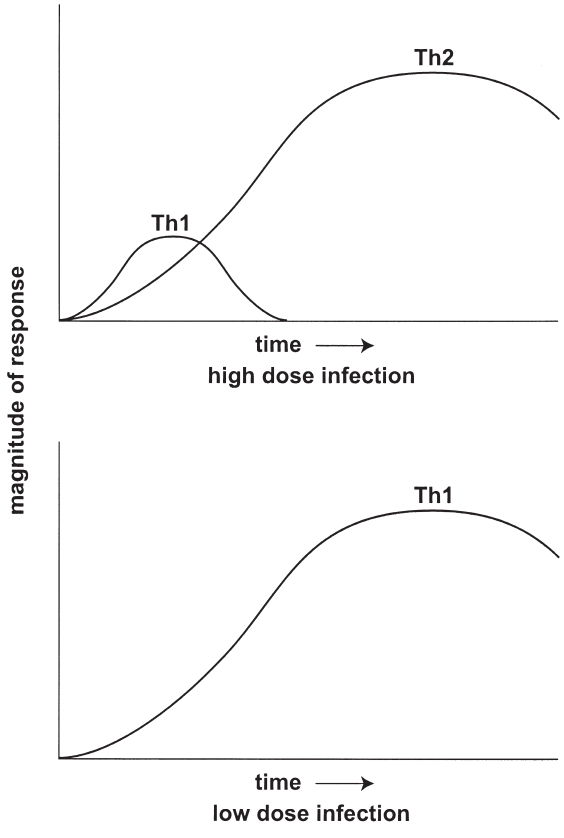

Figure 2. Primary response to infection [based upon ref. 62].

and are recognised by anti-q1, anti-q2, ... anti-qn CD4+ T cells. Coherence reflects the fact that the response to the different parts of an antigenic entity tend to be coordinately regulated with respect to the nature of their Th1/Th2 phenotype. Thus, there will be a tendency for the Th1/Th2 nature of the response to q1, q2, ... qn to be the same, i.e. either predominantly Th1, Th2 or mixed. Another example of coherence is seen in the antibody response. The class of antibody to a hapten, when raised by immunising with a hapten carrier-conjugate, tends to be the same as the class of antibody generated against the carrier. Coherence can be accepted as a 'fact' reflecting immune class regulation, which is primarily what we shall do in this article, or one can try to understand its mechanistic basis. From the latter perspective, we can briefly note the following. The activation of pTh cells specific for q1, q2, ... qn is interdependent, because anti-q3 CD4+ T cells can influence the activation of anti-q2 pTh cells, and vice versa, through mechanisms involving linked recognition. For example, we have noted above that TsDTH cells act via the recognition of linked epitopes. In addition, if the threshold hypothesis is valid, as we believe, the Th1/Th2 nature of the progeny of an anti-q3 pTh cell following activation depends upon the number and state of activation of other CD4+ T cells specific for other q peptides. These mechanisms can provide an explanation for coherence [24]. In addition, the Th1/Th2 nature of concurrent responses, occurring in the same lymphoid organ, to different and non-cross-reacting antigens, are apparently independently determined [47]. Thus the Th1/Th2 nature of the response to the peptides q1, q2, ... qn, derived from Q tend to be similar, and are unaffected by the process determining the Th1/Th2 nature of the response to the peptides r1, r2,...rn, derived from the non-cross-reacting antigen R. This independence of responses seems to be important, in allowing the immune system to simultaneously mount the different kinds of response required to optimally contain different invaders. This independence can be abrogated when there are overwhelming infections [48–50].

Vaccination and treatment

Pathogen-specific or general approaches to achieving effective vaccination

One plausible and rational approach to achieving effective vaccination is to start by defining in some detail the mechanisms that underlie protection in resistant animals or people for a particular infectious disease. Such a definition may help to inspire approaches to achieving or ensuring a resistant state. A natural stage in this process is to define the 'protective antigens' against which immunity is required to gain resistance. Once defined, these antigens can be employed in vaccine formulations.

We shall explore the alternative approach, whether the design of vaccination and treatment strategies is feasible without an experimental definition of protective antigens. Such an approach may prove abortive, leading to its abandonment. However, should it be feasible, it offers the possibility of common vaccination strategies applicable against many different pathogens. It is partly for this reason that we took a virus, a bacterium and a family of protozoa as our candidate intracellular pathogens. The possibility of success of this approach is based upon the phenomenon of coherence. We hope that we can guarantee the right kind of protective immunity to the large majority of the antigens of a pathogen by appropriate imprinting, thereby guaranteeing the right kind of immunity against the protective antigens, without having to define what these protective antigens are. Moreover, given the genetic diversity of MHC genes, and the fact of MHC-restricted recognition of antigen by T cells, protective T cell epitopes, and possibly the antigens from which they are derived, are likely to be different in different people. Thus the pathway to effective vaccination through the definition of protective antigens seems complex.

The approach we explore should not be taken to imply that we do not consider the nature of the detailed mechanisms of pathogen containment to be of the greatest interest and importance. Knowledge of such mechanisms is likely to inspire novel means of preventing and treating disease. In this same spirit of exploring the broad approach, we shall not emphasize the different cytokines made by Th1, Th2 and other cells and their role in pathogen containment.

Problems for achieving universally efficacious vaccination

It is helpful at the outset to acknowledge the major recognised problems in achieving universally efficacious vaccination [51]. We shall later deal with problems associated with treatment.

The nature of the protective response

Observation has led us and many others to the working hypothesis that exclusive cell-mediated Th1 (CTL) responses are optimally protective for certain intracellular infections. However, this is sometimes questioned. Convincing evidence will consist in showing that a vaccination strategy that ensures the hypothetical immune correlates of protection upon natural infection does in fact result in protection. In other words, we need a successful vaccination strategy to prove beyond reasonable doubt what the real correlates of protection are, but we need to know what these correlates of protection are to rationally design an effective vaccination strategy. There is nothing one can do better, in these circumstances, than take the plunge: make a hypothesis concerning the immune corre-

lates, design a vaccination strategy that attempts to ensure these correlates are achieved following natural infection, and then undertake a field trial.

Imprinting upon the immune system to ensure a protective response

We need to know, even if we correctly guess the immune correlates of protection, how to achieve an imprint upon the immune system that guarantees this protective response. The existence of immune deviation, and the occurrence of cell-mediated and humoral locked states, as described above, suggests that imprinting is possible. One of the most exciting developments of recent years has been the achievement in various ways of effective imprinting in animal studies, particularly in the murine model of cutaneous leishmaniasis. Appropriate imprinting results in resistance [16]. We shall discuss these successes and their potential significance for human studies.

Genetic heterogeneity of the human population

Genetic polymorphisms are known to affect immune responses both quantitatively and qualitatively. How, in view of this, can we design a standard vaccination protocol that is effective in all people?

Impingement upon the immune system by environmental organisms that cross-react with the pathogen

One recognised potential problem in vaccination against tuberculosis is the occurrence of environmental mycobacteria that impinge upon the immune systems of the host population. This may be a more general problem that we discuss in the context of vaccination against tuberculosis. Environmental mycobacteria cross-react with *M. tuberculosis* and the mycobacteria that constitute BCG, the attenuated mycobacterial strain used to vaccinate against tuberculosis and leprosy. The effects of such environmental mycobacteria upon the immune system may interfere with vaccination [52]. We discuss below some mouse studies that support this possibility. It has been suggested, on the basis that environmental mycobacteria are more prevalent in countries closer to the equator, that the lower efficacy of protection observed in different BCG vaccination trials in such countries can be laid at the door of environmental mycobacteria. This type of correlation may be significant. However, so many variables between different trials could account for their different efficacies that relying on sole causes for explaining differences in the efficacies of different trials seems unwise.

The murine leishmaniasis system

There are many murine studies employing *Leishmania major* parasites, the protozoan that lives inside macrophages and causes human cutaneous leishmaniasis. When different strains of mice are infected with a million *L. major* parasites, different kinds of immune responses are generated, associated with different consequences. CBA, C3H and C57Bl mice produce a predominant and stable Th1 response and contain the parasites, and are hence called resistant. BALB/c mice produce a transient Th1 response, which declines as Th2 cells are generated. They suffer progressive disease, with an increasing parasite burden [11], and are called 'susceptible'. A number of simple manoeuvres carried out close to the time of infection render BALB/c mice resistant to this high-dose infection. These include giving neutralising anti-IL-4 antibodies [53], administering IL-12 [54] or giving anti-CD4 antibody resulting in partial depletion of CD4+ T cells [55, 56]. All these different approaches modulate the ensuing immune response towards the Th1 pole and result in increased resistance. These observations make it very plausible that the correlation between resistance and the generation of parasite-specific Th1 cells, and susceptibility and the generation of Th2 cells, is not merely fortuitous but causal. In addition, the administration of anti-IFNγ antibodies to normally resistant mice modulates the response towards a Th2 mode and makes the mice susceptible [57].

Infection of BALB/c mice with a low number, about a thousand parasites, results in an exclusive, Th1 response. With time, these mice become extremely resistant to a high-dose challenge and, as expected, this resistance is associated with a predominant Th1 response [16]. These studies with *L. major* were based upon those of Parish, described above, demonstrating the establishment of cell-mediated immune deviation to a protein antigen [15]. Similarly, BALB/c mice that contain a first infection with a high parasite number, due to the simultaneous administration of IL-12, become resistant to a normally pathogenic, subsequent high-dose challenge [58]. These observations show the dramatic consequences of Th1 imprinting. We argue later that evidence shows Th1 imprinting can occur in people against *Leishmania donovani*, responsible for visceral leishmaniasis [59].

The murine model of cutaneous leishmaniasis has also been useful in showing that the prevalence of different IgG subclasses of anti-parasitic antibody reflects the Th1/Th2 nature of the response. For example, in BALB/c mice, a very exclusive Th1 response does not result in detectable antibody, a predominant Th1 response (with a small Th2 component) in IgG2a antibody, a mixed Th1/Th2 response in a mixed IgG2a/IgG1 antibody response, and a predominant Th2 response in predominant IgG1 antibody [60]. Similar observations have been made following infection with *T. muris* [42]. These observations collectively suggest that the prevalence of different subclasses of IgG antibody might be employed to assess the Th1/Th2 nature of the immune response.

Low-dose vaccination strategy

BCG is an attenuated form of *M. bovis* that causes tuberculosis in cattle and sometimes in people. It has been used more than any other vaccinating agent worldwide, having been given to roughly two billion individuals. Various trials have shown BCG to provide variable protection against tuberculosis. This variability could have many sources: the strain of BCG employed, the age of the people vaccinated, the genetic constitution of the different populations involved as subjects in different trials, and environmental factors, such as the preponderance of environmental mycobacteria [52]. The famous Madras trial examined the efficacy of BCG vaccination in the late 1960s and early 1970s, and involved over a quarter of a million subjects. This trial showed overall no efficacy of protection. The doses of BCG used were chosen to be high but below that causing significant side effects [61].

A major problem in making progress in the field of human vaccination are the limitations imposed on experiments, for understandable ethical reasons. Suppose we wanted to test a vaccination strategy by a field trial. It is natural to suppose that we would have to have found valid solutions to the four problems listed above if the vaccination strategy is to be effective, because failure to address one problem is likely to result in unreliable protection. For this reason, developing tentative solutions to these problems one at a time through animal studies is worthwhile.

The dose dependence of the cell-mediated-Th1/antibody-Th2 nature of the immune response appears to hold for protein antigens [15], xenogeneic red cells [40], leishmania parasites [16], mycobacteria [44–46], simian immunodeficiency virus (SIV) [41] and a murine retrovirus [43]. This dose dependence for leishmania parasites holds even for different strains of parasite, different routes of infection, and different host strains of mice [62]. The salient features of this latter study illustrate some important facts that may allow the development of a standard and effective vaccination protocol in a genetically diverse population, and so will be briefly described. Given a particular strain of leishmania parasite and site or route of infection, a transition number of parasites, n_t, can be defined [62]. Infection with a number of parasites below n_t results in a stable cell-mediated Th1 response and containment of the parasite, whereas infection with a number above n_t results, in time, in a stable Th2 response and progressive parasite growth. The value of n_t depends on the host [63]. We found two strains of mice for which the value of n_t for a given parasite strain and site of infection differed by a millionfold. We found that not only are 'susceptible' BALB/c mice resistant to a low-dose infection, but 'resistant' CBA succumb to a sufficiently high-dose challenge. Figure 3 shows the ratio of IFN-γ/IL-4 produced by parasite-specific cells for five different situations, involving different hosts, different parasite strains and different routes of infection, and in which mice were infected with a low dose (below n_t) or a high dose (above n_t) of parasites in each of the five chosen circumstances. The patterns seen in these diverse situations are remarkably similar. The IFN-γ/IL-4 ratio is about 100- to a 1000-fold higher for the low- than for the high-dose infection in all cases at 8 weeks post-infection, reflecting the generation of predominant Th1 and Th2 responses, respectively [62]. Natural infection might be anticipated to result usually in a 'low dose infection', and therefore in resistance, but disease obviously occurs, for example when certain parasite strains can generate a Th2 response in 'susceptible hosts' even when the infection is small (see fig. 3). Second, components of saliva from the sandfly vector may adversely affect the outcome of infection by a few parasites, favouring the generation of a Th2 response [64]. The generality of the above finding on the dependence of the Th1/Th2 nature of the response on antigen dose might allow the design of a low-dose vaccination strategy that is universally efficacious. We consider BCG vaccination against tuberculosis to illustrate this. Suppose a dose of BCG is injected that is below the value of n_t for all members of a genetically diverse population. The BCG will, for a particular individual, be either at a level where it can generate a Th1 response and Th1 imprint, or be below the threshold level required to generate a Th1 response. In this latter case, the BCG will slowly replicate until it has reached the threshold required to generate Th1 cells and a Th1 imprint [51]. This vaccination process may not be effective in all individuals if the low dose is cleared in some individuals by innate defense mechanisms. A strategy to overcome this possibility is discussed elsewhere [51]. Low-dose BCG vaccination can generate a Th1 imprint in BALB/c mice [63], and low-dose BCG vaccination worked dramatically in cattle to prevent experimental tuberculosis [46]. Some evidence suggests that low-dose SIV infection might be able to protect macaques against a higher and normally pathogenic challenge of SIV [41, 65].

The stability of Th1 and Th2 imprinting

A remarkable feature of the Th1 imprinting achieved through low-dose infection is its stability. Challenge of such Th1-imprinted mice with a high number of parasites, which generates a highly dominant Th2 response in naive animals, results in an ever more dominant Th1 response over a period of at least 3 months, a considerable fraction of a mouse life [60]. This robustness is illustrated in figure 4. We have also found that exposure to high doses of parasite antigen results in a Th2 response and the generation of a Th2 imprint. Such mice infected with a low number of parasites, which are contained by naive mice due to a predominant Th1 response, now produce a Th2 response and suffer progressive infection [O. Ogunremi and P. A. Bretscher, unpublished observations]. Fig-

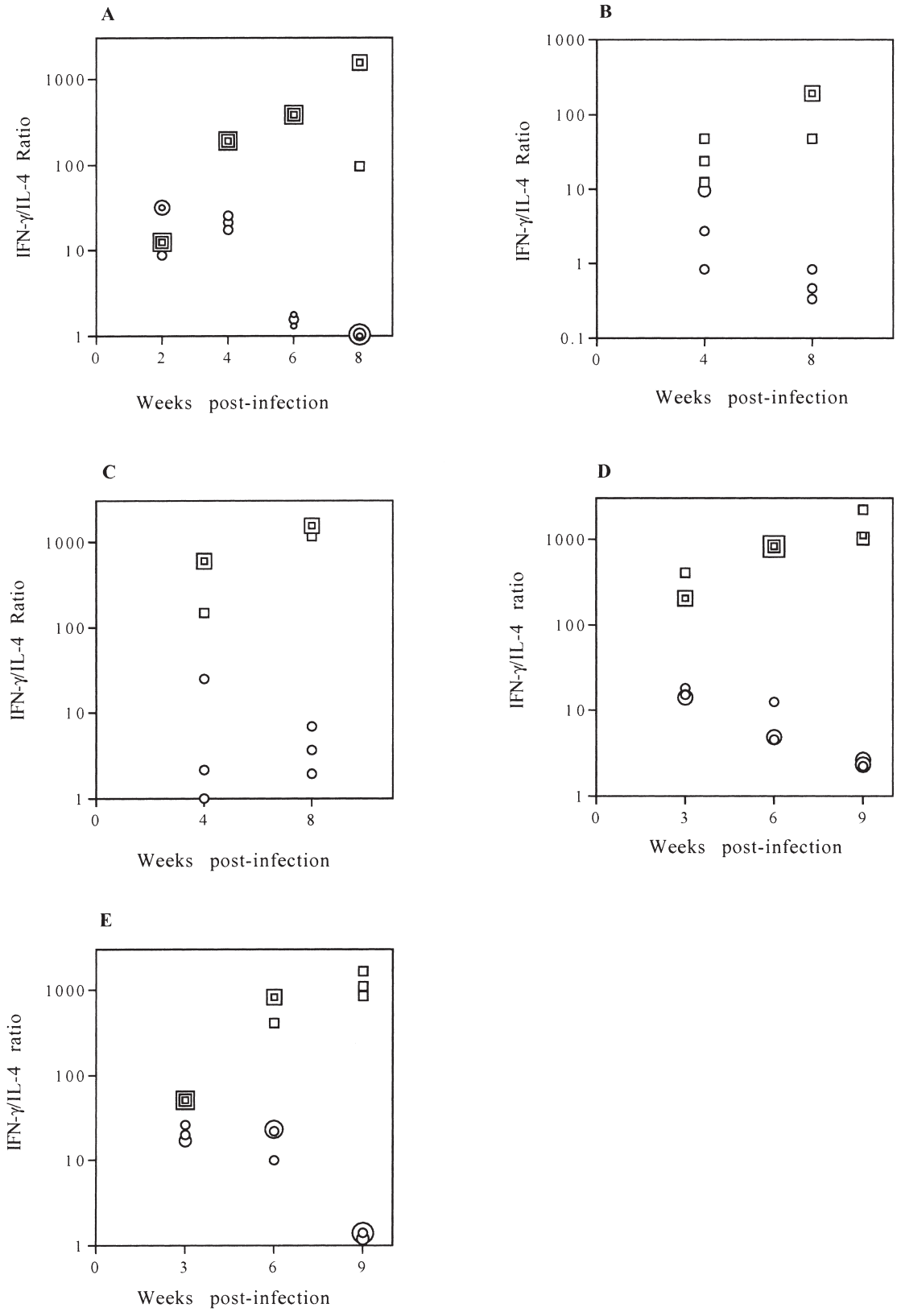

Figure 3. The ratio of IFN-γ to IL-4 production following infection with a relatively high (above n_t) and low (below n_t) number of parasites [based upon ref. 62, with permission]. In all cases, squares represent the ratio in mice given the relatively low dose of parasites, circles the ratio in mice given the relatively high dose of parasites. (*A*) *L. major* Friedelin parasites (5×10^2 and 10^7) given to BALB/c subcutaneously (s.c.) on the rump. (*B*) *L. major* NIH 173 parasites (33 and 10^4) given to BALB/c s.c. on the rump. (*C*) *L. major* NIH 173 parasites (10^4 and 5×10^8) given to CBA mice s.c. on the rump. (*D*) *L. major* NIH 173 parasites (33 and 5×10^7) given to A/J mice s.c. on the rump. (*E*) *L. major* NIH 173 parasites (33 or 10^8) given to CBA mice intravenously.

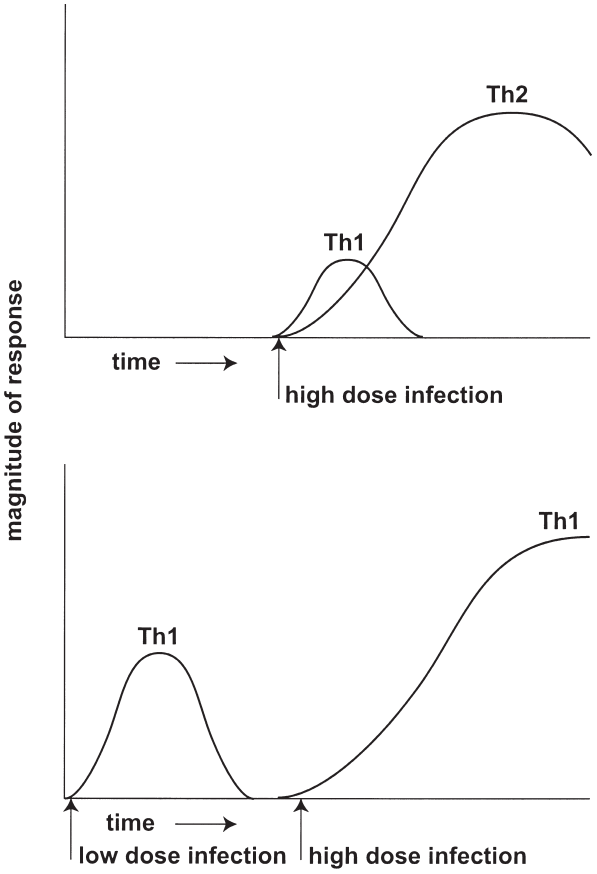

Figure 4. Th1 imprinting [based on ref. 60] The upper panel shows a primary, Th2 response following high-dose infection or administration of a high dose of antigen. The panel below illustrates the effect of pre-exposure to a low-dose infection resulting in a Th1 response on a subsequent high-dose challenge. Pre-exposure generates a Th1 imprint.

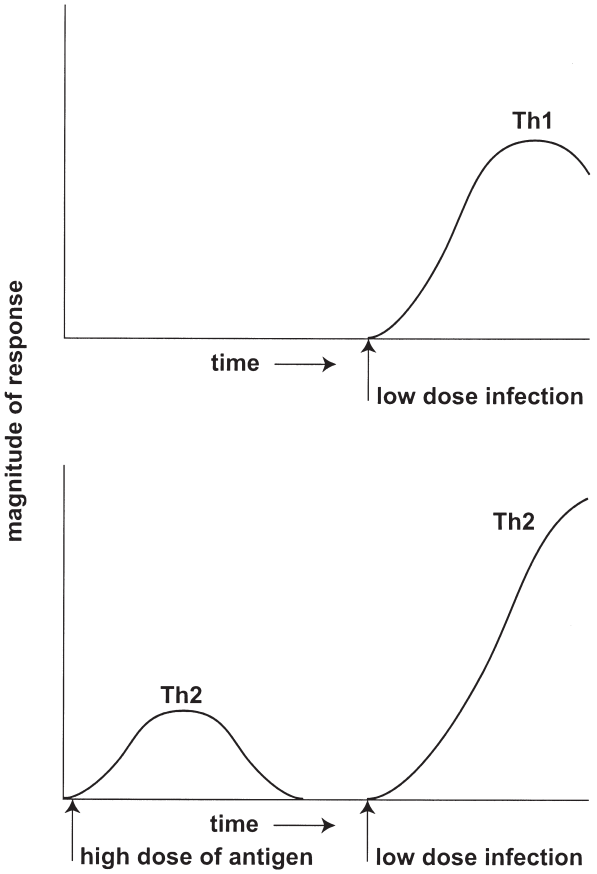

Figure 5. Th2 imprinting [based upon unpublished observations of O. Ogunremi and P. A. Bretscher]. The upper panel shows a primary, Th1 response following low-dose infection or administation of a low dose of antigen. The panel below illustrates the effect of pre-exposure to a high dose of antigen, resulting in a Th2 response, on a subsequent low-dose challenge. Pre-exposure generates a Th2 imprint.

ure 5 illustrates the generation and consequence of Th2 imprinting. These observations illustrate a potential consequence of inappropriate vaccination. Inappropriate immunisation may not only be ineffective but may be detrimental. As we can see, it can alter the consequences of a low-dose infection that is usually contained and is therefore usually not pathogenic.

The above two examples illustrate that both Th1 and Th2 imprinting can be stable. This suggests that any detrimental consequences of environmental mycobacteria on the vaccination process can be minimised by establishing an effective Th1 imprint before the environmental mycobacteria establish any detrimental Th2 imprint. These considerations lead to the suggestion that neonatal vaccination is advisable. One might wonder whether, around birth, the Th1/Th2 nature of the response shows a similar antigen dose dependence as in more mature individuals. Reassuringly, infection of neonatal mice with a low viral burden can induce exclusive cell-mediated responses, with higher burdens inducing antibody [43].

We have found that the Th1/Th2 nature of the response to BCG is dependent on the mycobacterial dose in the expected manner [44]. However, we found that older mice gave highly inconsistent, sporadic results. We also found that these older mice, even those we had not immunised, had anti-mycobacterial antibody. Interestingly, *M. gordonii* was isolated from their drinking water. We seem to have rediscovered environmental priming and its consequences on responses to BCG!

Universally efficacious strategy of vaccination

The considerations outlined above would suggest that neonatal infection with a very low dose of a slowly replicating agent such as BCG, too low to produce an immune response with a significant Th2 component in any member of the population, should in time generate a Th1 response and Th1 imprint in all members of the population. The establishment of this Th1 imprint neonatally would minimise the untoward effects of Th2 imprinting by envi-

ronmental mycobacteria. This is the basis for the neonatal low-dose BCG vaccination strategy against tuberculosis. We believe that the low-dose strategy might be widely applicable. One important consideration is the use of BCG or other vectors to establish Th1 imprints to various antigens such as viral proteins. We have studied the immune response to BCG vectors expressing the β-galactosidase gene. We observed responses anticipated from the phenomenon of coherence. Low-dose infection resulted in a Th1 response to both BCG and β-galactosidase, whereas infection with higher doses of the vector led to mixed responses to both BCG and the expressed protein. Low-dose infection caused Th1 imprinting [66]. These results hold promise for using BCG vectors to immunise against viruses such as HIV-1.

Pathogen/microbial persistence and immunological memory: implications for vaccination and control of reactivation disease

We have seen that BALB/c mice infected with a large number of parasites generate a predominant Th2 response and suffer progressive disease, whereas infection with low numbers results in a virtually exclusive Th1 response and containment of the parasite. Infection with numbers around the transition number, n_t, results in a few mice containing the infection, a few developing progressive disease, associated with predominant Th1 and Th2 responses, respectively, and most mice developing a large but stable lesion associated with a mixed Th1/Th2 response. We refer to this state as borderline disease, in analogy with borderline human leprosy, characterised as having a mixed cell-mediated/antibody response. Note that this range of different pathophysiological states can be generated in one mouse strain just by changing the number of parasites employed for infection.

An infection is established in the large majority of BALB/c mice given a low number of parasites, as judged by the recovery of parasites from the infected foot and draining lymph node a few weeks after parasite inoculation. Mice without any visible lesion but harbouring parasites are said to have a subclinical infection. The conventional wisdom is that mice cannot eliminate leishmania parasites and that humans, once infected, cannot eliminate *M. tuberculosis*. A number of observations led to the strong inference that some subclinically infected mice clear their infection of leishmania parasites. A more analytical system was developed, in which the transfer of spleen cells from subclinically infected mice to lightly irradiated recipients, challenged with a million parasites, leads to the containment of the parasites and often to their subsequent complete elimination, as assessed by a limiting-dilution test for parasites. This putative elimination of parasites is associated with loss of any detectable antiparasite response, loss of resistance to the parasite, and loss of parasite-specific memory T cells, as assessed by limiting-dilution analysis. These observations appear to provide compelling evidence for parasite elimination, or at least a reduction to such low levels that immunological memory decays as assessed by a variety of assays [J. Uzonna, D. Yurkowski, G. Wei and P. A. Bretscher, unpublished data].

Persistent infection maintains resistance but leaves the individual susceptible to reactivation disease, whereas elimination and consequent memory loss leaves the individual open to reinfection but not to reactivation disease. All disease states, whether due to a new infection or to reactivation disease, increase the size of the pathogen reservoir and are therefore disadvantageous to the host population.

The demonstration of immune elimination of pathogens and vaccinating agents is important from a variety of perspectives. First, as illustrated by the studies just described in murine cutaneous leishmaniasis, elimination results in loss of memory and resistance, with obvious implications for the long-term efficacy of vaccination. These observations in a natural system appear inconsistent with the idea, drawn from other studies, that the maintenance of memory T cells does not require direct or indirect stimulation through their T cell receptor [67]. Consider the consequences of this loss of memory in terms of BCG vaccination as an example. Clearance of BCG will result in loss of resistance, unless there is a continual reinforcement of Th1 imprinting from exposure to mycobacteria. In the absence of such naturally occurring exposure, either by pathogenic or environmental mycobacteria, the population must be periodically revaccinated to maintain resistance. Second, tuberculosis and cutaneous leishmaniasis are believed to result sometimes from loss of immune control of subclinical infections, due to suppression of the immune system, resulting in ‘reactivation disease’. This can occur through ageing or, for example, HIV-1 infection. If certain pathogens can indeed be eliminated, then one should be able to make use of such elimination to prevent reactivation disease. For example, if individuals have appropriate immunity to eliminate mycobacteria and are continually exposed to environmental mycobacteria, one would expect an individual exposed to *M. tuberculosis* to eliminate the pathogen with time, thus losing their susceptibility to reactivation disease [J. Uzonna, D. Yurkowski, G. Wei and P. A. Bretscher, unpublished data].

Treatment of disease

Many studies have been directed at curing disease in the mouse model of cutaneous leishmaniasis. Most of the ‘simple’ treatments appear to be effective only when given around the time of infection of mice with a high number of parasites that cause rapidly progressive dis-

ease. These 'simple' treatments include administration of anti-CD4 antibody that results in depletion of CD4+ T cells, or the administration of neutralising anti-IL-4 antibody, or the administration of IL-12 or of Th1-promoting bacterial DNA CpG sequences [68]. Although these are all interesting interventions, they do not appear to provide models for treating human disease, because they are only effective when administered around the time of infection, usually before lesions are apparent. Established lesions can be cured by 'complex' treatments, such as giving anti-CD4 antibody to achieve total depletion of CD4+ T cells and anti-IL-4 antibody, so that the new response generated on T cell regeneration is Th1 dominant. Another means is by giving antimonial drugs, which by itself causes some morbidity when used in the field to treat visceral leishmaniasis, together with IL-12. These 'complex' treatments are perhaps not very practical because, for example, the total depletion of CD4+ T cells will leave the treated individual susceptible to other infections. Some further studies have deliberately employed models of established infections corresponding to either borderline or very slowly progressing cutaneous leishmaniasis, with the idea that such models correspond more closely to most human disease [69]. In this case, simple treatments, such as the administration of anti-IL-4 antibody, or the partial depletion of CD4+ T cells, are found to deviate the response to the Th1 pole with a speedy and dramatic regression of the lesion. These studies provide some hope for the development of simple treatments for human disease [J. Uzonna and P. A. Bretscher, Eur. J. Immunol., in press]. We shall discuss other forms of potential treatment of human disease in the next section.

Human diseases

Tuberculosis

Immune parameters distinguishing between the immune state of patients and healthy contacts seem much clearer in some diseases than in others. For example, visceral leishmaniasis patients usually have very poor skin sensitivity to leishmanial antigens, whereas their healthy contacts show skin sensitivity [11]. The presence of IgG4 and IgG3, and high-level expression of IgG1 anti-parasite antibody, distinguishes the immune state in patients from that in healthy contacts. Further observations suggest that this isotype pattern reflects the fact that patients have a greater Th2 component to their immune response [59]. Contrast this with findings in tuberculosis. Here, most healthy contacts and most tuberculosis patients show skin sensitivity to mycobacterial antigens. In the case of tuberculosis, there is convincing evidence that, on average, patients have more IgG antibody than healthy contacts, but there is much overlap between the values seen in healthy contacts and in patients [5, 6]. Other studies suggest that there are more mycobacterial-specific IL-4-producing T cells in patients than healthy contacts, but this is again only evident at the population level rather than at the level of the individual [6]. We looked at the levels of anti-mycobacterial IgG subclasses in tuberculosis patients and healthy contacts to determine if their antibody subclasses differ, because IgG subclasses are surrogate markers of the Th1/Th2 nature of the immune response in mice and in humans infected with the pathogen responsible for visceral leishmaniasis. In one study, levels of IgG3 and IgG4 were undetectable in many patients, but IgG1 and IgG2 were more prevalent. IgG1 titres in about two-thirds of the tuberculosis patients were in the same range as those in the healthy contacts, but about one-third of the patients had significantly higher levels of antibody. Based on the study of human visceral leishmaniasis just discussed, these patients with high IgG1 values plausibly had a greater Th2 component to their response than the other tuberculosis patients and healthy contacts. Partly based upon these results, we have suggested that there are two different immunopathological forms of tuberculosis, reflecting two different types of immune system failure. In 'type I tuberculosis', patients are envisaged to mount qualitatively the same kind of response as healthy contacts (IgG1 low), but the response is too weak to contain the infection at tolerable levels. Observations show that virtually exclusive Th1 responses can exist, a situation that may require inhibition of the generation of Th2 cells, as indicated above. Such responses may not be damaging if low and effective in containing an infection, but should they not be strong enough, the size of the infection will increase and the magnitude of the response may also increase considerably, leading to significant bystander damage and hence to a pathological state. In 'type II tuberculosis', the response has a Th2 component (IgG1 high) and is ineffective for this reason [J. N. Menon, V. H. Hoeppner, A. Judd, M. V. Kanchana, D. D. Marciniuk, C. A. Power et al., unpublished observations]. The attractions of this proposal are threefold. First, the existence of two kinds of tuberculosis explains why no immunological parameter can discriminate between the immune state in most healthy contacts and most patients at the level of the individual. In addition, others have suggested that there is a spectrum of disease states in tuberculosis as in leprosy [70]. Second, the most prevalent view that all TB patients have a Th2 component to their response is not only difficult to reconcile with current observations, but makes the spontaneous cures of tuberculosis seen before the advent of antibiotics somewhat puzzling. It seems to be a general rule that the immune response evolves (without clinical interventions) to have a greater Th2 component with time, if it evolves at all. If a patient has an immune response against *M. tuberculosis* that is ineffective due to a Th2 component, the disease is unlikely to resolve spontaneously. Studies in mice suggest that type I tuberculosis

may resolve. Resistant CBA mice given ten million *L. major* parasites mount a predominant Th1 response. This infection gives rise to a very substantial lesion that resolves over a period of about 2 months, presumably because it takes considerable time for the Th1 response to become sufficiently substantial to contain such a large infection [16]. This infection model might correspond to resolving type I tuberculosis. Third, Koch in the 1890s developed a form of antigen therapy that appears to have had diverse results [4]. While some patients benefited, others clearly worsened after treatment [71, 72]. We suggest that the differential results of Koch's therapy may relate to the two immunopathological forms of TB proposed earlier. Antigen therapy in patients with type II disease would increase the antigen load and this would be expected to drive the response to have a greater Th2 component and hence be detrimental. However, antigen treatment may well be beneficial in type I disease if the size of the mycobacterial burden is limiting the size of the response. The potential importance of different types of immunological failure leading to disease is that different types of failure are likely to require different forms of immunotherapy.

AIDS

Studies of prostitutes [73, 74], healthcare workers exposed to contaminated blood [75], sexual partners of HIV-infected individuals [76, 77] and children born to HIV-infected mothers [78–80] have shown that individuals exposed to HIV but whose infection is undetectable often have cell-mediated immune responses to HIV antigens in the absence of detectable antibody. These studies suggest that some individuals may be able to control or even eliminate an HIV infection and that cell-mediated immunity in the absence of antibody is the optimally protective immune response. Protection appears to require both CD4+ and CD8+ cells. CD8+ T cell anti-HIV effector functions are diverse, and include lysis of virus-infected cells and production of anti-viral factors such as the beta-chemokines [81]. Recent studies have revealed that CD4+ T helper cells are required for maintenance of CTL function [82–85]. Although many HIV-infected individuals have high numbers of HIV-specific CTLs in later stages of disease, these cells are less effective or non-functional due to the lack of CD4+ helper T cell function [85]. Thus, not only does effective HIV immunity require CTLs, but also Th1 cells able to provide help to these CTLs. Therefore, induction of CTLs and Th1 cells is likely to be critical for providing protection to HIV.

There is evidence to suggest that, as in the studies with leishmania and BCG, initial low-level exposure to HIV can lead to a Th1/CTL response which is effective in controlling and perhaps clearing the HIV infection. Individuals who have been exposed to HIV via a needlestick often have HIV-specific CD4+ T cell responses following exposure but no HIV-specific antibody [75]. These individuals remain HIV-negative, suggesting the cell-mediated immune response may have cleared the virus or contained it at very low levels. Other studies carried out using SIV infection of macaques have shown that low doses of SIV given intrarectally induce a Th1/CTL response which protects these animals from further exposure to the virulent virus [41, 65]. Furthermore, studies using replication-defective SIV have shown that both virulence and protection correlate with replication rate [86]. Attenuated viruses must replicate enough to induce immunity but not so fast that they rapidly generate a high viral load, leading to an immune response with a significant Th2 component and hence progressive disease. Such attenuated viruses can induce CTL responses and protection from AIDS caused by heterologous virus challenge [86, 87]. Similarly, naturally attenuated viruses have been isolated from long-term survivors with non-progressive HIV infection [88]. We suggest that these attenuated viruses are able to induce a Th1/CTL response and consequent protection because they are slow growing, resulting in chronic low-level antigen stimulation as in the BCG and leishmania models. Due to the inherent dangers of using attenuated viruses for vaccination, we suggest that low-dose immunisation with recombinant BCG expressing HIV antigens may be an appropriate alternative for achieving an effective AIDS vaccine.

Visceral leishmaniasis and leprosy

One study strongly supports the view that people can be Th1 imprinted for resistance to *L. donovani.* The immune state of four groups of people was studied: unexposed individuals, healthy contacts, patients and individuals cured through administration of antimonial drugs. As outlined above, patients could easily be distinguished from healthy contacts by the presence of high levels of IgG1, and the presence of significant levels of IgG3 and IgG4 antibodies and lack of skin sensitivity to leishmanial antigens. Most interestingly, the immune status of the cured individuals was indistinguishable from that of the healthy contacts, showing that treatment had modulated the immune response from the state in patients to that observed in healthy contacts [59]. Treatment very often results in resistance to reinfection, a finding accounting for these and other observations. This is interesting from two perspectives. First, humans can appparently be Th1 imprinted, resulting in resistance. Second, the question is raised concerning the basis of this beneficial modulation of this immune response upon treatment.

Antimonial drugs are known to kill parasites, but they are in addition highly toxic. One possible mechanism by

which they might modulate the response towards a Th1 pole is by killing parasites and hence reducing the amount of antigen, in analogy with the finding that low doses of antigen favour the primary generation of Th1 responses. Some in vitro studies support this possibility [89]. In addition, successful treatment and modulation of the response was not achieved with some patients in one study, and these patients were found to harbour drug-resistant parasites. Treatment with a second-line anti-parasite drug led to both successful modulation and treatment. This provides compelling evidence that the effect of the drug is due, in some degree, to its effect in killing parasites. Three manoeuvres around the time of infection can modulate a primary Th1/Th2 or Th2 response to become Th1 dominant: lowering the antigen dose, partial depletion of CD4+ T cells or administration of neutralising anti-IL-4 antibody. The efficacy of these manoeuvres is understandable in terms of our knowledge of the nature of the decision criterion controlling the Th1/Th2 nature of the primary immune response. Their parallel efficacy in modulating mixed Th1/Th2 responses towards a Th1 pole suggests that the decision criterion controlling ongoing mixed responses has essential features in common with those governing primary responses. This knowledge should provide a rational approach to treatment.

Studies in leprosy patients show that lepromatous leprosy patients have a higher ratio of mycobacterial IgG1/IgG2 compared to tuberculoid leprosy patients. Treatment of lepromatous patients in general results in a significant reduction in this ratio reflecting a modulation of the immune response towards the Th1 pole [J. N. Menon, P. Saunderson, D. Kidane and P. A. Bretscher, unpublished observations].

Tumour immunology

Studies in the 1950s and 1960s led to the view that syngeneic tumours could usually be contained by cell-mediated but not humoral immunity [26]. Two types of observation appeared to have wide applicability in many tumour systems, and have provided grounds for optimism to those so inclined. The first was the phenomenon of concomitant immunity. Mice given a lethal dose of tumour cells could, under certain circumstances, resist a second lethal challenge even as the first tumour implant progressed. Transfer studies from such tumour-bearing animals showed that protective immunity was induced, but that too little was generated too late. Moreover, the studies of North and his colleagues showed that the initial generation of protective cell-mediated immunity is not sustained due to the generation of tumour-specific CD4+ T cells that suppress the activity/generation of the protective cell-mediated response. These suppressor cells may be Th2 cells [24]. It is worth noting in passing the similarlity in this picture to that following HIV infection, where the initial response is protective, and illness starts to develop once seroconversion occurs.

The second general finding that nourishes the optimist is the ability to establish immunological resistance to syngeneic tumours. Most interestingly, the excision of the tumour at approximately the time of optimal expression of cell-mediated immunity often results in long-term resistance to a normally lethal challenge [90]. This virtually ubiquitous procedure for attaining a tumour-specific resistant state is known as excision priming. The removal of the bulk of the tumour just at the time of optimal expression of protective cell-mediated immunity, thereby halting the evolution of the anti-tumour response to a Th2 mode by removing most of the antigen, has led to the suggestion that this procedure is analogous to low-dose vaccination and Th1 imprinting [24]. This proposal would in essence equate excision priming with low-dose vaccination. Indeed, recent correlative observations in humans suggest that progressive cancer may sometimes be associated with tumour-specific Th2 responses, and regression with Th1 responses [91–95]. In general, there might be at least two types of immune system failure in cancer as in tuberculosis: too small a response of the correct type, or a response ineffective because of a detrimental Th2 component. The definition of genes and proteins responsible for the generation of peptides recognised by 'tumour-specific' T cells, in animal and human systems, as exemplified by the work of Boon and colleagues [96] should allow systematic approaches to beneficially regulate the immune response to tumours.

Conclusions

We have explored in this article the possibility of developing general strategies for the prevention and treatment of chronic diseases caused by intracellular pathogens or oncogenic events. We have made these proposals by attempting to recognize general and broad features of the regulation governing the immune response. Such approaches should, to the extent that they are valid, be applicable to the prevention and treatment of diverse diseases, which is why we considered a virus, a bacterium and a protozoan as our prototypic, slowly growing intracellular pathogens. We are excited by the possibility that the broad scheme developed may provide an appropriate framework for the design of strategies to prevent and treat diverse diseases. Time will tell.

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