

Vaccines against intracellular infections requiring cellular immunity

Robert A. Seder* & Adrian V. S. Hill†

*Clinical Immunology Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA

†Molecular Immunology Group, Institute of Molecular Medicine, Nuffield Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK

Vaccines against a variety of infectious diseases represent one of the great triumphs of medicine. The immune correlates of protection induced by most current vaccines seem to be mediated by long-lived humoral immune responses. By contrast, there are no currently available vaccines that are uniformly effective for diseases such as HIV, malaria and tuberculosis, in which the cellular immune response might be crucial in mediating protection. Here we examine the mechanisms by which long-lived cellular immune responses are generated and maintained *in vivo*. We then discuss current approaches for vaccination against diseases in which cellular immune responses are important for protection.

The origins of acquired immunity against infectious disease were observed more than 2,000 years ago. In 430 BC, Thucydides described the plague of Athens in his historical account of the Peloponnesian war:

It was with those who had recovered from the disease that the sick and the dying found most compassion. These knew what it was from experience, and had now no fear for themselves; for the same man was never attacked twice — never at least fatally.

At the end of the eighteenth century, Jenner's observation that vaccination with cowpox virus protected individuals from smallpox provided the scientific basis for the concept of vaccination. Today, vaccines for a variety of bacterial and viral diseases have had a major impact worldwide in reducing both morbidity and mortality. The main reason for the success of current vaccines is that, for most viral and bacterial infections, primary protection is thought to be mediated by a long-lived humoral immune response through the production of antibodies. It is noteworthy that such responses are readily induced by many different vaccine formulations. By contrast, for infections such as tuberculosis, malaria and HIV, which now bring about a substantial proportion of the deaths worldwide from infection, there are no uniformly effective vaccines. Furthermore, although humoral immunity could be important in preventing infection by HIV and certain stages of malaria infection, it is the cellular immune response that is central to the mediation of protection in all of these diseases. The focus of this review is our understanding of how long-lived cellular immune responses can be induced *in vivo* after vaccination.

Acquired cellular immunity

The acquired cellular immune response is composed of CD4⁺ and CD8⁺ T cells. CD4⁺ T cells recognize proteins — commonly referred to as antigens — after they have been processed by a specific group of cells termed antigen-presenting cells. CD4⁺ T cells recognize antigens that have been processed through the exogenous pathway by antigen-

presenting cells (such as dendritic cells, macrophages and B cells) expressing major histocompatibility complex (MHC) class II molecules (Fig. 1). After the recognition of antigen in association with MHC class II molecules, CD4⁺ T cells become activated, and differentiation can occur into functional subsets termed T helper 1 (T_H1)-type and T helper 2 (T_H2)-type cells. The distinction of these subsets is based on their production of a variety of proteins called cytokines. The signature cytokines for T_H1 and T_H2 cells are interferon (IFN)- γ and interleukin (IL)-4, respectively. T_H1 cells, through their production of IFN- γ , mediate the killing of organisms responsible for a variety of intracellular infections. For many intracellular infections, the induction of a functional T_H1 response is crucially dependent on another cytokine, IL-12, which is produced by antigen-presenting cells such as macrophages or dendritic cells after exposure to the pathogen at the initiation of the immune response. Thus, in response to many intracellular infections, IL-12 is the inducer cytokine of T_H1 cells, and IFN- γ is the effector cytokine that mediates protection. CD8⁺ T cells recognize antigens that are processed and presented by cells expressing MHC class I molecules. CD8⁺ T cells also mediate their effector function through the production of cytokines such as IFN- γ and tumour necrosis factor (TNF)- α and/or through a direct cytolytic mechanism (Fig. 1). The mechanism of cytolytic killing can be mediated by the release of granule contents such as perforin and granzyme from CD8⁺ T cells. In addition, CD8⁺ T cells can kill cells by a process of Fas-mediated lysis.

The role of T_H1 and CD8⁺ T cells in protective immunity

In many experimental rodent models of intracellular infection, which include malaria and *Mycobacterium tuberculosis*, mice made deficient in genes for IFN- γ or IL-12 or functionally depleted of CD8⁺ T cells have all been shown to have an increased susceptibility to infection, with accelerated mortality. Moreover, humans with specific mutations in their receptors for IFN- γ or IL-12 have been shown to have enhanced susceptibility to mycobacterial disease. Together, these results provide strong evidence that for tuberculosis, malaria and other parasitic infections including *Leishmania major*, T_H1 and/or CD8⁺ T-cell responses are the effector mechanisms required for protective immunity.

Finally, there is evidence that CD8⁺ T cells also have a crucial role in mediating protection against HIV infection in both primates and humans. Thus, the development of a successful vaccine for these diseases will be facilitated by a thorough understanding of how cellular immune responses are generated and maintained *in vivo*.

Cellular memory

The major hallmarks of the immune response are discrimination between self and non-self, and immune memory. Whether it relates to cellular or humoral immunity, immune memory is the conceptual basis for how vaccines work. With regard to cellular memory, CD4⁺ and CD8⁺ memory T cells differ from naive T cells in the following manner. First, memory cells express specific cell-surface markers that can denote prior activation. Second, memory cells are qualitatively different from naive T cells and seem to have less stringent requirements for activation. In this regard, memory cells respond to a previously encountered antigen or pathogen (secondary response) more vigorously and faster^{1,2} than naive T cells. Furthermore, memory cells can be further divided into 'resting' and 'effector' cells. Effector memory cells are usually large, whereas resting memory cells are small. Effector CD4⁺ T cells (T_H1 cells) readily secrete cytokines, whereas effector CD8⁺ T cells have direct cytolytic activity *ex vivo*. By contrast, resting memory CD4⁺ or CD8⁺ T cells need to be stimulated with antigen before producing cytokines or acquiring cytolytic activity, respectively. Finally, a recent study³ suggests that differential expression of the chemokine receptor CCR7 segregates human memory T cells into two functionally distinct subsets. CCR7⁺ cells have been referred to as 'central' memory cells and express lymph-node homing receptors but lack immediate effector function. CCR7⁻ cells display immediate effector function and have been referred to as 'effector' memory cells. Thus, the differential expression of CCR7 on T cells permits the existence of two separate pools of memory cells that are dynamic and can serve to mediate effector function in response to infection at different sites. It should also be noted that, after activation, CCR7⁺ cells differentiate into CCR7⁻ cells and acquire effector function. These results support a linear differentiation model in which naive T cells first develop into central memory (CCR7⁺) and then proceed to effector memory cells (CCR7⁻)³. Two crucial factors that are most likely to regulate the generation, maintenance and effector aspects of T-cell memory responses and the transition from CCR7⁺ to CCR7⁻ cells are antigen and cytokines.

Requirement for antigen in maintaining cellular memory

In recent years there has been tremendous interest in whether antigen is required to sustain memory T cells. This issue has been controversial in that some reports show that antigen is required to maintain memory cells⁴⁻⁶, whereas others find that memory CD8⁺ T cells can be maintained in an antigen-independent manner⁷⁻⁹. It is currently our view, on the basis of all the evidence, that antigen is not essential to sustain memory cells *in vivo*. Moreover, in terms of cytokine production (namely IFN-γ) for both CD4⁺ and CD8⁺ T cells^{8,9}, rapid responsiveness can also be maintained from long-lived memory T cells in the absence of antigen. But as it relates to immune protection in diseases in which CD8⁺ T cells are important (such as malaria and HIV) there is strong evidence that antigen might be required to mediate a protective response. This could relate to the factors discussed below and is supported by the following results. First, in the rodent model of malaria infection after inoculation, parasites quickly enter the liver and infect hepatocytes. There is a marked expansion in parasite growth over the next several days, and then parasites exit the liver and enter the circulation, where they infect red blood cells. Because parasites infect hepatocytes, which express MHC class I, they are targets for CD8⁺ T-cell cytolytic activity. In this regard in a rodent model of malaria infection, after vaccination with DNA encoding specific malarial antigens, protection was induced in a manner dependent on CD8⁺ T cells and IFN-γ (ref. 10). These and other results clearly establish the importance of CD8⁺ T cells in protection against malaria in this rodent model. In addition, vaccination with

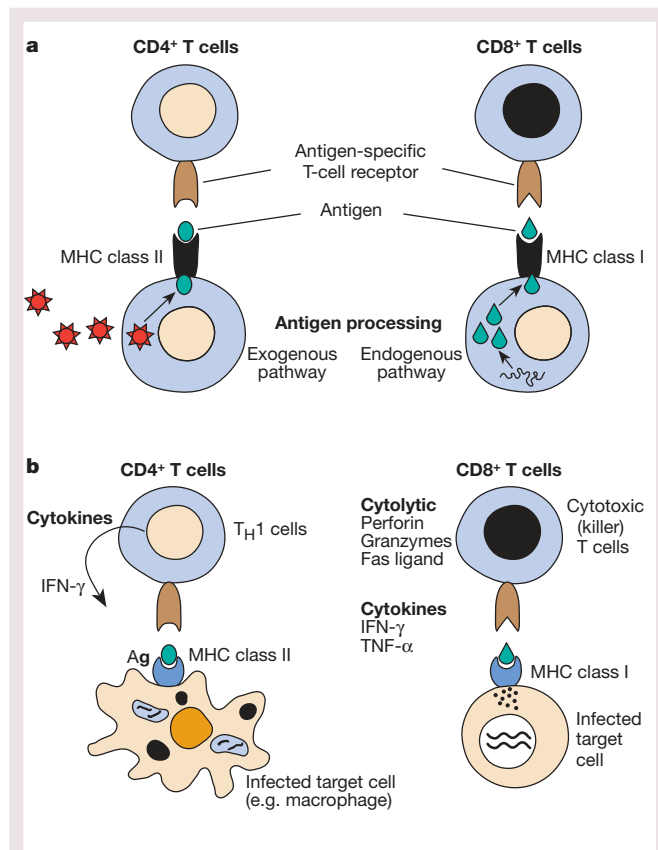


Figure 1 Mechanism of T-cell activation and effector function. **a**, Mechanism of antigen processing and recognition by T cells. **b**, Effector function of T_H1 and CD8⁺ T cells. Ag, antigen.

live irradiated sporozoites confers protection in rodents, primates and humans against a challenge with live unirradiated sporozoites¹¹. Protective immunity after vaccination with irradiated sporozoites in rats was abrogated by treatment with a drug that eliminated the pre-existing parasites in the liver before re-challenge¹². Thus, although the immune mechanism by which irradiated sporozoites are mediating their protective effect is not clear at present, these data suggest that persistent antigen might be required to maintain protection. Moreover, one could speculate that in sporozoite-vaccinated mice, persistent antigen (that is, sporozoites) was required to sustain CD8⁺ T-cell responses that were mediating protection. In infection with HIV, there is also strong correlative evidence that enhanced CD8⁺ T cell cytolytic activity is associated with a reduced viral load in infected primates^{13,14} and in patients with HIV¹⁵. Moreover, by monitoring the frequency of HIV-specific CD8⁺ T cells using MHC class I-specific tetramers, it was shown that treatment of patients with highly active antiretroviral therapy (HAART) caused a decrease in the number of antigen-specific CD8⁺ cells^{15,16}. This potential ability of antigen (viral load) to sustain CD8⁺ cytolytic activity is the basis for current clinical trials in which HIV-infected patients on HAART have structured interruptions of their treatment to expose the immune system briefly to antigen and thus sustain the cytolytic response^{16,17}. It is hoped that this strategy will generate a persistent cellular immune response that can serve to limit viral replication even after therapy ceases.

With regard to CD4⁺ T cell memory (T_H1 memory), it has been demonstrated that after the adoptive transfer of short-term T_H1 lines that were generated *in vitro*, cells are maintained *in vivo* for several months in the absence of antigen or even MHC class II molecules¹⁸. Thus, from these results, it is clear that antigen is not required for sustaining a pool of 'resting' memory T_H1 cells that were generated *in*

vitro. Moreover, in a different adoptive transfer model, it was also demonstrated that CD4 memory T cells could be sustained in the absence of antigen¹⁹. In this study, it was of interest that not all memory T cells expressed IFN- γ when assessed *ex vivo*. These results suggested that there could be a heterogeneous population of memory CD4⁺ T cells. One type of memory cell could rapidly produce IFN- γ after stimulation, whereas the other type of memory cell required additional differentiation for effector function. These results might be analogous to the delineation of effector/memory cells (CCR7⁻) and central/memory cells (CCR7⁺), respectively, as discussed above. What has not been well defined is whether persistent antigen is required for the maintenance of protective levels of memory/effector T_H1 cells that are generated *in vivo* after immunization. Some insight into the importance of antigen in maintaining immunity can be gained from observations in the course of human clinical disease. As noted above, T_H1 cells have a clear role in mediating protection in rodent models of *M. tuberculosis* and cutaneous infection with *Leishmania*. In most people with tuberculosis or cutaneous infection with *Leishmania*, once infected individuals have resolved their initial infection they remain resistant to reinfection. Moreover, reactivation of tuberculosis can occur long after the resolution of primary infection in immunocompromised subjects, suggesting the existence of persistent live mycobacteria. In addition, persistent antigen has been documented in cutaneous infection with *Leishmania* many years after primary infection²⁰. Taken together, these results suggest that persistent antigen might be important in sustaining immunity in diseases that require T_H1 cells and/or CD8⁺ cytolytic cells. Finally, although persistent antigen might be important for long-term immunity that is induced by primary infection or by vaccination for the diseases discussed here, there is also substantial evidence indicating that too much antigen can markedly impair T-cell immune responses. This could occur quantitatively by deletion of activated cells through antigen-induced cellular death (apoptosis) and/or qualitatively by inducing unresponsiveness of the T cell (clonal anergy). Thus, although the persistence of antigen might be important in conferring immune protection after vaccination, the amount of antigen present might also be a crucial factor in optimizing long-term T-cell immunity.

Requirement for cytokines in maintaining cellular memory

Another factor besides antigen that is crucial for generating and sustaining memory T cells is the role of cytokines. For CD8⁺ T cells, there is evidence that IFN- α acting through IL-15 might be important in sustaining memory CD8⁺ T-cell responses²¹. As noted above, CD4⁺ T cells require IL-12 for differentiation into functional T_H1 cells. Moreover, there is now evidence that IL-12 might also be required to sustain memory/effector T_H1 cells sufficient to mediate a biological outcome. This was shown in a mouse vaccine model of infection with *L. major* in which it was demonstrated that continuous IL-12 was required to sustain T_H1 cells sufficient to control infection after challenge²². Finally, T_H1 responses have also been shown to mediate protection against *M. tuberculosis*. Currently, the live attenuated Bacille Calmette–Guérin (BCG) strain, which is an inducer of IL-12 and T_H1 responses, does provide some protection against systemic disease early in childhood. By contrast, the efficacy of BCG vaccination against pulmonary disease occurring later in life is quite variable. Although there are many reasons why BCG might not be uniformly effective against pulmonary disease, one potential explanation is that, over time, there is a sterilization of BCG. This would limit the ability both to provide a source of antigen and to induce cytokines such as IL-12.

Thus, for both T_H1 and CD8⁺ T-cell memory responses, the persistence of antigen might not be required for the maintenance of ‘resting’ memory CD4⁺ or CD8⁺ T cells but might be crucial for sustaining a pool of ‘effectors’ sufficient to provide protection for those diseases that require cellular immunity. Moreover, cytokines (such as IL-12) might also be required for the maintenance of a pool of memory/effector T_H1 cells sufficient for protection. The exact

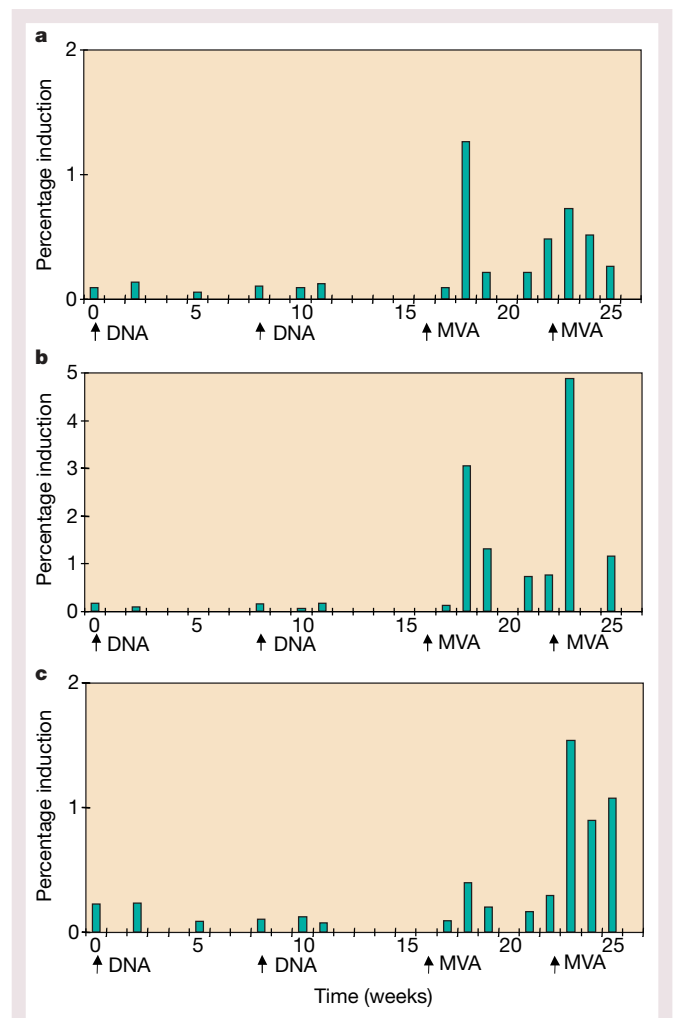


Figure 2 Induction of CD8⁺ T cells in three macaque monkeys (a–c) with the use of a heterologous prime–boost immunization regime (modified from ref. 46). Macaques were immunized with a plasmid DNA vaccine encoding a SIV CD8 T-cell epitope restricted by the MHC molecule Mamu-A*01 at weeks 0 and 8, and then immunized with a recombinant modified vaccinia Ankara (MVA) vaccine encoding the same epitope at weeks 16 and 22. The time course (in weeks) of induction of CD8 T cells specific for that epitope was measured by using an MHC–peptide tetramer; induction is expressed as the percentage of total peripheral blood CD8⁺ T cells. Prime–boosted animals showed higher responses than macaques immunized twice with MVA alone⁴⁶.

nature of these requirements depends on additional factors highlighted below.

Immune protection — thresholds, time and place

Thresholds

Most current vaccines require multiple immunizations for the induction of sufficient antibody to mediate protective immunity. In animal models of diseases requiring cellular immunity, there also seems to be a certain threshold of effector T_H1 or CD8⁺ T cells that is required for protection. For example, in rodent malaria a threshold level of about 400 IFN- γ -secreting peptide-specific CD8⁺ T cells per million splenocytes is required to protect against sporozoite challenge²³. In addition to the magnitude or threshold of the cellular response, it is also important to understand the ontogeny of the immune response after immunization or infection. In this regard, the induction of antigen-specific CD4⁺ and CD8⁺ T cells *in vivo* proceeds through three phases. First, after immunization or exposure to natural infection, there is an initial expansion phase in which there is a substantial increase in the frequency of antigen-specific cells^{24,25}.

Table 1 Comparative analysis of various vaccine formulations

		DNA vaccine	Live attenuated	Killed protein subunit	Live vector (e.g. poxvirus)
Immune response					
Humoral	B cells	++	+++	+++	++
Cellular	CD4 ⁺	+++ T _H 1*	± T _H 1	± T _H 1	+
	CD8 ⁺	++	+++	—	+++
Antigen presentation	MHC class	I and II	I and II	II	I and II
Memory					
Humoral		+++	+++	+++	++
Cellular		++	+++	±	++
Manufacturing					
Ease of development and production		++++	+	++	+++
Cost		+++	+	+	++++
Transport/storage		+++	+	+++	+
Safety		+++†	++‡	++++	++

*T_H2 responses can be induced by gene-gun immunization in mice.

†Data available only from phase I trials.

‡Live/attenuated vaccines may be precluded for use in immunocompromised patients and certain infections such as HIV.

This expansion phase, which usually occurs during the first week, is followed by the death phase, in which more than 90% of the activated cells undergo activation-induced apoptosis²⁶. For CD8⁺ T cells, the initial frequency of antigen-specific cells seems to be proportional to the antigenic load. In general, live infection induces a greater increase in the frequency of antigen-specific cells than immunizations with specific peptide, DNA vaccines or recombinant non-replicating viruses encoding the specific antigen²⁴. Moreover, it has been suggested that the persistence of antigen²⁷ and/or the amount of antigen²⁸ also affects qualitative aspects of CD8⁺ T-cell memory/effector responses. Once the initial expansion and death phases have occurred, the third phase of the response — the memory phase — can be stably maintained over a long period, even in the absence of persistent antigen^{1,7,29}. This memory phase of the response in the presence or absence of persistent antigen is likely to be heterogeneous, consisting of ‘central’ and ‘effector’ memory cells. The requirement for antigen in sustaining these specific memory cell types is not clear at present and is likely to have a large effect on whether or not immune protection is achieved, depending on the particular type of infection. Finally, the magnitude of the memory phase is generally determined by the size of the initial clonal burst induced by immunization³⁰. Thus, for protective memory CD8⁺ T-cell responses for diseases such as HIV and malaria that might require a high number of memory/effector cells, it is desirable to use a vaccine regimen that provides a sufficient antigenic load to maximize the initial burst size, so that a threshold of memory cells is maintained that will be protective.

Time and place

Currently used vaccines work by either preventing infection or preventing disease. For certain infections such as HIV, which can remain latent for long periods before progressing to clinical infection, the prevention of any infection by a vaccine would be optimal. Prevention of primary infection is probably mediated by a humoral immune response. By contrast, CD8⁺ T cells exert their effector function by recognizing antigen that has been processed in the context of MHC class I, and act to kill infected cells and to prevent a further spread of the infection. Thus, although CD8⁺ T cells might not themselves be able to prevent infection, they still could have a beneficial role in limiting or preventing disease, depending on how efficient they are in killing all of the infected cells. Furthermore, the effectiveness of the cellular immune response depends on where exposure to the pathogen occurs, and on whether the immune response exists within that site or needs to migrate from an immune organ. This crucial factor of time and place is determined by the specific infection. Thus, for rapidly replicating viruses such as influenza, it has been shown that the ability to generate long-term memory CD8⁺ T cells after primary infection is not sufficient to prevent disease in the lungs

after re-challenge³¹. In this study, even though the frequency and kinetics of the secondary CD8⁺ T-cell responses in the lungs were enhanced, it still took several days for cells to localize to the lungs from the mediastinal lymph nodes. With regard to HIV infection, it has recently been shown that after the intravaginal inoculation of rhesus monkeys with simian immunodeficiency virus (SIV), it was possible to find CD3⁺ T cells in the cervix that contained viral RNA by 3 days after infection³². These results imply a relatively narrow time window during which effector CD8⁺ T cells need either to be present at the mucosal site or to migrate quickly from a systemic site. At present, generating a high frequency of effector CD8⁺ cytolytic responses at mucosal sites has been difficult and represents a daunting challenge for vaccines. Nevertheless, the ability to generate a pool of ‘systemic’ memory CD8⁺ T cells that can be activated after exposure to HIV might not prevent infection or kill all infected cells, but could still limit the initial viral set point after acute infection, providing some measure of protection³³. In contrast with certain viral infections in which infection, latency and clinical disease can be established relatively quickly, time might be less of a limiting factor for protection against certain parasitic or mycobacterial infections. For malaria, the pre-erythrocytic liver stage of infection lasts for about 7 days in humans, providing a potentially long window for the action of effector T cells. It is important to note that complete killing of all parasites must occur within the liver to prevent release into the circulation and the infection of red blood cells. Finally, for pulmonary tuberculosis or cutaneous leishmanial infection, the time between initial exposure and clinical disease can be several weeks or longer. In these infections, it might be possible for a low frequency of ‘resting’ memory T cells that are generated after vaccination to develop into effector T cells that can control and prevent clinical disease. The challenges that lie ahead for vaccine development will be not only to optimize the qualitative and quantitative cellular immune response but also to induce responses directly at sites of infection.

Vaccines inducing cellular immunity to intracellular pathogens

All currently licensed vaccines (killed or inactivated, whole-cell, recombinant protein, or live attenuated) generate humoral immune responses. A potential shortcoming of non-live vaccines is their relative inefficiency in generating cellular immune responses (T_H1 or CD8⁺ T-cell responses), thus limiting their application for those diseases requiring cellular immunity (Table 1). Moreover, although certain adjuvants (used to enhance the potency of the vaccine) given with non-live vaccines have been shown to induce T_H1 responses, it has been difficult to find any adjuvant that can facilitate CD8⁺ T-cell induction in humans. In contrast, live attenuated vaccines permit the efficient MHC class I presentation of antigen that can stimulate CD8⁺ T-cell responses. The ability of live attenuated vaccines to induce

T_H1 -specific responses in humans, however, is less well characterized and can depend on the specific pathogen. Live vaccines also have the advantage of antigen persistence. From a practical standpoint, and also from one of safety, attenuated whole-organism vaccines raise several issues that can preclude their widespread application for certain diseases (namely HIV and malaria). Thus, the demonstration that DNA vaccines could induce both humoral and broad cellular responses (cytolytic and T_H1 responses)³⁴ in small animals provided hope that they could become the future vaccine of choice for infections requiring cellular immunity in humans³⁵. This optimism must be tempered by the fact that the induction of humoral and cellular immune responses after vaccination of humans with DNA has been disappointing in comparison with the rodent models^{36–38}. This has prompted investigators to try to optimize the immunogenicity of plasmid DNA vaccines themselves and/or to use DNA vaccines in combination with other vaccines to enhance immunity.

In terms of optimizing DNA vaccines in humans, several approaches are being taken to improve immunogenicity. In this regard, one approach has focused on specific nucleotide sequences (CpG motifs) within the plasmid. In both mice and humans, CpG oligodeoxynucleotides have been shown to stimulate multiple types of immune cell, leading to enhanced T_H1 and cytolytic $CD8^+$ T-cell responses^{39–42}. Moreover, recent work has shown that the immunostimulatory activity of CpG oligodeoxynucleotides can be blocked by certain non-CpG motifs⁴³. Thus, there is currently an effort to determine whether the addition of specific stimulatory CpG motifs, and the deletion of inhibitory ones, within the plasmid backbone can improve their immunogenicity. With regard to combining DNA with other types of vaccine, there have been several studies evaluating prime–boost strategies with vaccines based on live virus or on protein. Live virus vectors themselves typically generate stronger cellular immune responses than do DNA vaccines in small animals, yet clinical trials of poxvirus and adenovirus vectors have generally yielded fairly small cellular immune responses. The enhanced immunogenicity of a heterologous prime–boost immunization strategy, by using DNA priming and boosting with a recombinant poxvirus vector that encodes the same foreign antigens, has been demonstrated in several infectious diseases^{23,44}. In mice and in primates (Fig. 2), substantial enhancement of $CD8^+$ T-cell responses was observed, and the immunization order was crucial for maximal immunogenicity. Several poxviruses, such as modified vaccinia virus Ankara (MVA) and fowlpox, and also replication-defective adenoviruses, have this capacity to boost a primed $CD8^+$ T-cell response substantially^{45,46}. Clinical trials of this approach for malaria with MVA as a boosting agent are in progress. Finally, although the current approach of using a DNA prime and a MVA boost is clearly important in optimizing the magnitude of the $CD8^+$ response, it is not clear how long the enhanced responses will be maintained after the MVA boost. But this raises the issue of whether continued boosting will be required for sustaining a sufficient number of cells to confer protection.

Another method for optimizing the qualitative and quantitative immune response after vaccination is to use an adjuvant. Currently, the only widely used adjuvant in humans is alum, which improves antibody production but has little effect on T_H1 or $CD8^+$ T-cell responses. A novel adjuvant consisting of QS21, monophosphoryl lipid A (MPL) and an oil-in-water emulsion, SBAS2, has been shown to generate both strong antibody responses and T_H1 -type responses in combination with a protein antigen in malaria. This 'RTS,S' vaccine has shown impressive but relatively short-lived protective efficacy in sporozoite challenge studies and is currently undergoing field trials⁴⁷.

Perspective

Various new vaccination strategies for inducing strong cellular immune responses are now reaching clinical trials. Several of these are being evaluated for diseases such as malaria or HIV or in a

therapeutic setting where efficacy can be assessed in small studies. In addition, as noted above, other immunization approaches will probably be required for inducing effective mucosal immunity and for generating different types of T-cell response such as $CD1$ - and other non-classically restricted T cells. Although not all of these new approaches will generate sufficiently durable immunity for useful immunoprophylaxis, even the short-term potent induction of T effector cells might be of value for therapeutic vaccination. Furthermore, even if vaccines fail to induce long-term cellular immune responses, if exposure occurs within a short time of vaccination — a period during which the immune response is robust — protection could be achieved, and then life-long immunity would be possible as a result of the boosting effect of the live infection. The progress in developing vaccines for cellular immunity will also be enhanced by recent advances in the monitoring of T-cell responses by using sensitive enzyme-linked immunospot techniques and new tetrameric reagents. These tools provide excellent opportunities for the identification of immunological correlates of protection after vaccination. As a whole, our increased understanding of the immune correlates of protection as well as of the requirements for generating and sustaining cellular immune responses provides a framework for successful vaccine development against those diseases that require cellular immunity. □

- Ahmed, R. & Gray, D. Immunological memory and protective immunity: understanding their relation. *Science* **272**, 54–60 (1996).
- Dutton, R. W., Bradley, L. M. & Swain, S. L. T cell memory. *Annu. Rev. Immunol.* **16**, 201–223 (1998).
- Sallusto, F., Lenig, D., Forster, R., Lipp, M. & Lanzavecchia, A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* **401**, 708–712 (1999).
- Gray, D. & Matzinger, P. T cell memory is short-lived in the absence of antigen. *J. Exp. Med.* **174**, 969–974 (1991).
- Bachmann, M. F., Kundig, T. M., Hengartner, H. & Zinkernagel, R. M. Protection against immunopathological consequences of a viral infection by activated but not resting cytotoxic T cells: T cell memory without 'memory T cells'? *Proc. Natl Acad. Sci. USA* **94**, 640–645 (1997).
- Kundig, T. M. et al. On the role of antigen in maintaining cytotoxic T-cell memory. *Proc. Natl Acad. Sci. USA* **93**, 9716–9723 (1996).
- Lau, L. L., Jamieson, B. D., Somasundaram, T. & Ahmed, R. Cytotoxic T-cell memory without antigen. *Nature* **369**, 648–652 (1994).
- Tanchot, C., Lemonnier, F. A., Perarnau, B., Breitau, A. A. & Rocha, B. Differential requirements for survival and proliferation of $CD8$ naive or memory T cells. *Science* **276**, 2057–2062 (1997).
- Murali-Krishna, K. et al. Persistence of memory $CD8$ T cells in MHC class I-deficient mice. *Science* **286**, 1377–1381 (1999).
- Doolan, D. L. et al. Circumventing genetic restriction of protection against malaria with multigene DNA immunization: $CD8^+$ cell-, interferon γ -, and nitric oxide-dependent immunity. *J. Exp. Med.* **183**, 1739–1746 (1996).
- Guebrenne-Xabier, M., Schwenk, R. & Krzych, U. Memory phenotype $CD8^+$ T cells persist in livers of mice protected against malaria by immunization with attenuated *Plasmodium berghei* sporozoites. *Eur. J. Immunol.* **29**, 3978–3986 (1999).
- Scheller, L. F. & Azad, A. F. Maintenance of protective immunity against malaria by persistent hepatic parasites derived from irradiated sporozoites. *Proc. Natl Acad. Sci. USA* **92**, 4066–4068 (1995).
- Matano, T. et al. Administration of an anti- $CD8$ monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. *J. Virol.* **72**, 164–169 (1998).
- Schmitz, J. E. et al. Control of viremia in simian immunodeficiency virus infection by $CD8^+$ lymphocytes. *Science* **283**, 857–860 (1999).
- Ogg, G. S. et al. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* **279**, 2103–2106 (1998).
- Kalams, S. A. et al. Levels of human immunodeficiency virus type 1-specific cytotoxic T-lymphocyte effector and memory responses decline after suppression of viremia with highly active antiretroviral therapy. *J. Virol.* **73**, 6721–6728 (1999).
- Ortiz, G. M. et al. HIV-1-specific immune responses in subjects who temporarily contain virus replication after discontinuation of highly active antiretroviral therapy. *J. Clin. Invest.* **104**, R13–R18 (1999).
- Swain, S. L., Hu, H. & Huston, G. Class II-independent generation of $CD4$ memory T cells from effectors. *Science* **286**, 1381–1383 (1999).
- Garcia, S., DiSanto, J. & Stockinger, B. Following the development of a $CD4$ T cell response *in vivo*: from activation to memory formation. *Immunity* **11**, 163–171 (1999).
- Schuback, A. et al. Detection of *Leishmania* DNA by polymerase chain reaction in scars of treated human patients. *J. Infect. Dis.* **178**, 911–914 (1998).
- Zhang, X., Sun, S., Hwang, L., Tough, D. F. & Sprent, J. Potent and selective stimulation of memory-phenotype $CD8^+$ T cells *in vivo* by IL-15. *Immunity* **8**, 591–599 (1998).
- Gurunathan, S., Prussin, C., Sacks, D. L. & Seder, R. A. Vaccine requirements for sustained cellular immunity to an intracellular parasitic infection. *Nature Med.* **4**, 1409–1415 (1998).
- Schneider, J. et al. Enhanced immunogenicity for $CD8^+$ T cell induction and complete protective efficacy of malaria DNA vaccination by boosting with modified vaccinia virus Ankara. *Nature Med.* **4**, 397–402 (1998).
- Oehen, S., Waldner, H., Kundig, T. M., Hengartner, H. & Zinkernagel, R. M. Antivirally protective cytotoxic T cell memory to lymphocytic choriomeningitis virus is governed by persisting antigen. *J. Exp. Med.* **176**, 1273–1281 (1992).

25. McHeyzer-Williams, M. G. & Davis, M. M. Antigen-specific development of primary and memory T cells *in vivo*. *Science* **268**, 106–111 (1995).
26. Razvi, E. S. & Welsh, R. M. Apoptosis in viral infections. *Adv. Virus Res.* **45**, 1–60 (1995).
27. Ochsenbein, A. F. *et al.* A comparison of T cell memory against the same antigen induced by virus versus intracellular bacteria. *Proc. Natl Acad. Sci. USA* **96**, 9293–9298 (1999).
28. Opferman, J. T., Ober, B. T. & Ashton-Rickardt, P. G. Linear differentiation of cytotoxic effectors into memory T lymphocytes. *Science* **283**, 1745–1748 (1999).
29. Mullbacher, A. The long-term maintenance of cytotoxic T cell memory does not require persistence of antigen. *J. Exp. Med.* **179**, 317–321 (1994).
30. Hou, S., Hyland, L., Ryan, K. W., Portner, A. & Doherty, P. C. Virus-specific CD8⁺ T-cell memory determined by clonal burst size. *Nature* **369**, 652–654 (1994).
31. Flynn, K. J. *et al.* Virus-specific CD8⁺ T cells in primary and secondary influenza pneumonia. *Immunity* **8**, 683–691 (1998).
32. Zhang, Z. *et al.* Sexual transmission and propagation of SIV and HIV in resting and activated CD4⁺ T cells. *Science* **286**, 1353–1357 (1999).
33. Lifson, J. D. *et al.* The extent of early viral replication is a critical determinant of the natural history of simian immunodeficiency virus infection. *J. Virol.* **71**, 9508–9514 (1997).
34. Ulmer, J. B. *et al.* Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* **259**, 1745–1749 (1993).
35. Seder, R. A. & Gurunathan, S. DNA vaccines — designer vaccines for the 21st century. *N. Engl. J. Med.* **341**, 277–278 (1999).
36. Wang, R. *et al.* Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. *Science* **282**, 476–480 (1998).
37. Le, T. P. *et al.* Safety, tolerability and humoral immune responses after intramuscular administration of a malaria DNA vaccine to healthy adult volunteers. *Vaccine* **18**, 1893–1901 (2000).
38. Calarota, S. *et al.* Cellular cytotoxic response induced by DNA vaccination in HIV-1-infected patients. *Lancet* **351**, 1320–1325 (1998).
39. Cho, H. J. *et al.* Immunostimulatory DNA-based vaccines induce cytotoxic lymphocyte activity by a T-helper cell-independent mechanism. *Nature Biotechnol.* **18**, 509–514 (2000).
40. Cowdery, J. S., Chace, J. H., Yi, A. K. & Krieg, A. M. Bacterial DNA induces NK cells to produce IFN- γ *in vivo* and increases the toxicity of lipopolysaccharides. *J. Immunol.* **156**, 4570–4575 (1996).
41. Klinman, D. M., Yi, A. K., Beaucauge, S. L., Conover, J. & Krieg, A. M. CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ . *Proc. Natl Acad. Sci. USA* **93**, 2879–2883 (1996).
42. Sato, Y. *et al.* Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. *Science* **273**, 352–354 (1996).
43. Krieg, A. M. *et al.* Sequence motifs in adenoviral DNA block immune activation by stimulatory CpG motifs. *Proc. Natl Acad. Sci. USA* **95**, 12631–12636 (1998).
44. Schneider, J. *et al.* Induction of CD8⁺ T cells using heterologous prime-boost immunisation strategies. *Immunol. Rev.* **170**, 29–38 (1999).
45. Kent, S. J. *et al.* Enhanced T-cell immunogenicity and protective efficacy of a human immunodeficiency virus type 1 vaccine regimen consisting of consecutive priming with DNA and boosting with recombinant fowlpox virus. *J. Virol.* **72**, 10180–10188 (1998).
46. Hanke, T. *et al.* Effective induction of simian immunodeficiency virus-specific cytotoxic T lymphocytes in macaques by using a multiepitope gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen. *J. Virol.* **73**, 7524–7532 (1999).
47. Stoute, J. A. *et al.* A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **336**, 86–91 (1997).

Correspondence and requests for materials should be addressed to R.A.S. (e-mail: rseder@niaid.nih.gov).