Antibodies, viruses and vaccines

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OPINION

Antibodies, viruses and vaccines

Dennis R. Burton

Neutralizing antibodies are crucial for vaccine-mediated protection against viral diseases. They probably act, in most cases, by blunting the infection, which is then resolved by cellular immunity. The protective effects of neutralizing antibodies can be achieved not only by neutralization of free virus particles, but also by several activities directed against infected cells. In certain instances, non-neutralizing antibodies contribute to protection. Several viruses, such as HIV, have evolved mechanisms to evade neutralizing-antibody responses, and these viruses present special challenges for vaccine design that are now being tackled.

Vaccines have been enormously effective in preventing human diseases caused by viruses. For example, it is estimated that up to 300 million people died from smallpox in the first three quarters of the twentieth century, whereas no one has died from the disease since 1978 owing to an eradication programme based on mass vaccination[1]. However, despite their efficacy, we do not have a clear understanding of how vaccines work. T cells and antibodies are central to protection, and immunological memory in some form is required, but there are many uncertainties and controversies. For example, the relative importance of cell-mediated and antibody responses in resisting viral infection is hotly debated. Opinions range from the view that antibodies are required primarily to control bacterial, rather than viral, infection and are dispensable for the control of some viral infections[2] to the view that antibodies are the only identified agent of successful vaccine protection[3,4] (R. M. Zinkernagel, personal communication). Nowhere have the uncertainties been felt more than in the HIV-1 vaccine field, in which efforts were focused initially on antibody responses elicited by subunit protein (SUBUNIT VACCINES)[5], but were later switched to T-cell responses elicited by a range of viral proteins[6–9].

How important are antibodies for vaccine-mediated protection? How do they operate? In this article, I approach these problems by considering the antiviral activity of antibodies at increasing levels of complexity. First, I review our current knowledge of the activities of antibodies *in vitro*. Second, I consider the activities of antibodies *in vivo*, as determined by passive-transfer studies, and relate them to the *in vitro* activities. Third, I discuss antibody activity in the complex context of vaccination. In addition, I consider some of the problems in eliciting protective antibodies, together with some potential solutions.

Antiviral activities of antibodies *in vitro*

In principle, antibodies can act against both free virus and infected cells, as shown in FIG. 1. Probably the most marked antiviral activity of antibody and the activity that is most important for antibody-mediated protection *in vivo* is the neutralization of free virus particles. Neutralization has been defined as "the loss of infectivity which ensues when antibody molecule(s) bind to a virus particle, and usually occurs without the involvement of any other agency. As such this is an unusual activity of antibody paralleled only by the inhibition of toxins and enzymes"[10]. The mechanisms of neutralization have been debated over the years. Prominent hypotheses have been that viruses are neutralized extracellularly by the binding of one or a few antibody molecules; that conformational changes in envelope or capsid molecules are crucial; or that viral inactivation by antibody can occur after entry to infected cells by, for example, blocking virus uncoating[10]. We have argued recently[11] in favour of a simple occupancy model, essentially as proposed initially by Macfarlane Burnet in 1937 (REF. 12). According to this model, neutralization occurs when a fairly large proportion of available sites on the virion are occupied by antibody, which leads to the inhibition of virus attachment to host cells or interference with the entry (fusion) process. The relatively large size of the antibody molecule, approximately similar to that of a typical viral envelope spike, is proposed to be crucial. A marked linear relationship between the surface area of a virus and the number of antibody molecules that are required to bind to the virus for neutralization supports this proposal. An important prediction of the 'occupancy' or 'coating' model is that the neutralizing efficacy of an antibody should be related to its affinity for antigen on the virion surface. So, a vaccine should aim to elicit antibodies of the highest affinity for virion surface antigen. It should be realized, however, that there remains considerable disagreement in the area and, furthermore, that different mechanisms might operate for different viruses under different conditions.

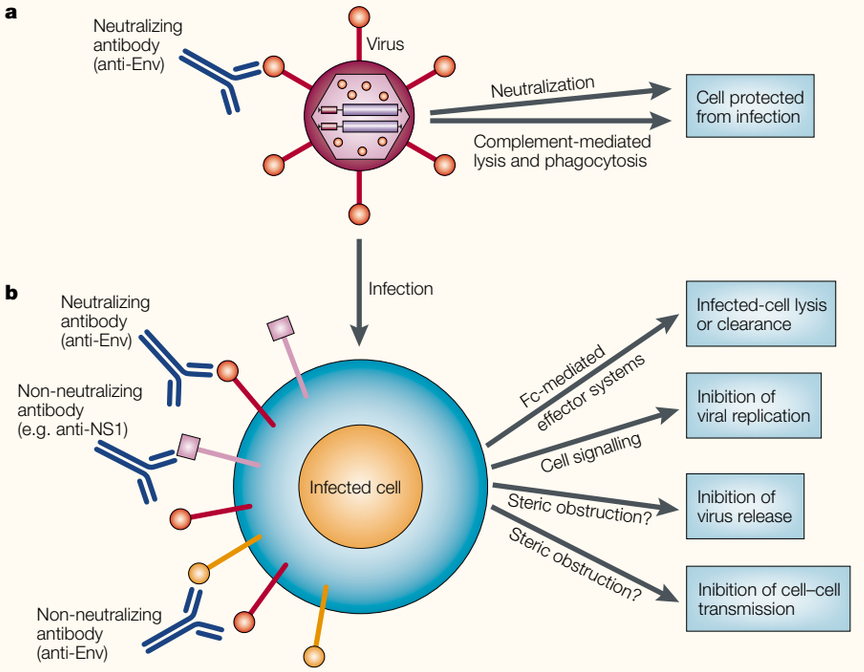

Figure 1 | **The antiviral activities of antibodies. a** | Activities against free virus (an enveloped virus is shown). Neutralizing antibodies probably act primarily by binding to the envelope protein (Env) at the surface of the virus and blocking infection (neutralization). They can also trigger effector systems that can lead to viral clearance, as discussed in the text. **b** | Activities against infected cells. These activities can be mediated by both neutralizing and non-neutralizing antibodies. Neutralizing antibodies bind to the same proteins on infected cells as on free virus. Non-neutralizing antibodies bind to viral proteins that are expressed on infected cells but not, to a significant degree, on free virus particles. Examples include altered forms of Env protein and certain non-structural (NS) proteins, such as NS1 of dengue virus. The binding of neutralizing and/or non-neutralizing antibodies to infected cells can lead to clearance of such cells or the inhibiton of virus propagation as shown. Modified from REF. 11.

Antibody Fc-mediated effector systems can affect antibody activity against free virus particles in several ways[13]. First, the activation of complement by antibodies that are bound to virus particles and the deposition of complement components on the virion surface can enhance neutralization. The occupancy model proposes that this is due to an increased coating of molecules on the virion, which prevents productive binding of the virion to the target cell. Second, complement activation can lead directly to virolysis. Third, Fc and complement receptors can bind antibody- and/or complement-coated virions, which leads to phagocytosis followed by inactivation of the virion in an intracellular compartment of the phagocyte. This process has been described *in vitro* for the picornavirus foot-and-mouth disease virus (FMDV) and it is believed to be important *in vivo* for protection against FMDV[14]. In FIG. 1, an enveloped virus that has functional spikes is shown to bind neutralizing antibodies exclusively. However, if antigen is present at a relatively low density on the surface of a virion, it could, in principle, bind antibody without resulting in neutralization. Such non-neutralizing antibody could, nevertheless, trigger complement-dependent virolysis or phagocytosis.

The binding of antibody to infected cells, as well as to free virus particles, can mediate several antiviral activities (FIG. 1). Fc-mediated effector systems can lead to cell lysis or clearance by antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). The inhibition of viral replication inside cells by the binding of antibodies to viral molecules that are expressed at the membrane of the cells, presumably through signalling mechanisms, has also been described, particularly for viral infection of neurons[15,16]. Antibodies can inhibit the release of viruses from infected cells[17] and the cell–cell transmission of viruses[18,19]. There is some evidence that antibody is less effective against infected cells than against free virions. For example, it has been reported that higher concentrations of neutralizing antibody are required to inhibit cell–cell transmission than to inhibit infection by free virions[18,20]. Similarly, a higher concentration of antibody has been shown to be associated with effective CDC and ADCC than with neutralization[21]. Neutralizing antibodies tend to be effective against infected cells because they bind to envelope molecules that are presented on infected cells as well as virions. However, non-neutralizing antibodies might also be effective against infected cells by binding to molecules that are expressed on infected cells, but not virions — for example, the NS1 protein of Dengue virus[22].

Polymeric immunoglobulin A and IgM can mediate the intracellular neutralization of viruses. These antibodies are actively transported across the mucosal epithelium after binding to the polymeric immunoglobulin receptor (PIGR) and might, during transport, come into contact with and neutralize transcytosing with viruses[23–27].

Finally, antibody can enhance viral infection *in vitro* under certain conditions, generally in the presence of subneutralizing concentrations of neutralizing antibodies. Enhancement is mediated by Fc receptors in some cases, such as Dengue virus[28–30], but not in other cases, for which enhancement is observed with antibody Fab fragments[31,32]. The occupancy model of neutralization that is described above explains the enhancement of infection as an effect that occurs at low occupancy of virion sites in the presence of permissive cells — for example, those bearing Fc receptors. As the concentration of antibody increases, coating of the virus increases, which eventually results in neutralization. Evidence for the importance of antibody-mediated enhancement *in vivo* is very sparse, even for the often-quoted example of infection with Dengue virus[33].

Antiviral activities of antibodies *in vivo*

The classical approach to determining the protective activities of antibodies *in vivo* is to transfer passively immune sera or monoclonal antibodies to a naive animal, challenge with virus and observe the outcome. This approach has shown consistently — for many different viruses, animal models and challenge routes — a good correlation between the protection that is achieved *in vivo* and antibody or serum neutralizing activity as measured *in vitro*[11]. It should be realized that this does not necessarily mean that neutralization is the mechanism of protective activity. Neutralizing antibodies, as noted above, are likely to be those that bind most effectively to free virions and to virus-infected cells (at least for many enveloped viruses), so that, in principle at least, any of the mechanisms of antiviral activity that are shown in FIG. 1 could operate in protection.

Generally speaking, protection is achieved when the neutralizing antibody titre in the serum of the animal at the time of virus

challenge is relatively high, usually of the approximate order of 1 in 100 for a 90% titre. In other words, the serum of the animal can be diluted 100-fold and 90% neutralization still be achieved *in vitro* (with higher dilutions for lower titres). This indicates that considerable apparent over-capacity with regard to neutralization is required to achieve protection. In some instances, protection can be described as sterile, in that there is no evidence of viral replication after challenge. Serum neutralizing-antibody titres (80%) of 1 in 380 or greater provide sterile protection in the lungs of cotton rats challenged with respiratory syncytial virus (RSV)[34], and titres of 1 in 400 (90%) and 1 in 38 (99%) provide sterile protection against challenge of macaques with chimaeric simian immunodeficiency virus (SIV)/HIV (SHIV)[35,36]. In other instances — such as challenge with lymphocytic choriomeningitis virus (LCMV) in a mouse model[37] and with Ebola virus in a guinea-pig model[38] — high titres of neutralizing antibody do not provide sterile protection, but do prevent disease. In most instances, however, it has not been established whether protection is sterile.

There are several possible explanations for the apparent over-capacity of neutralization activity that is required for protection in many animal studies. If one assumes that neutralization is the dominant protective mechanism, then for some viruses, animal models or challenge routes, it might be necessary for protection that antibody 'mops up' essentially every virus particle, which would require an excess of antibody. By the same assumption, the protective activity might occur in a tissue site that has a lower antibody concentration than that of serum, which would also lead to an apparent over-capacity. Alternatively, protection might require an additional activity of neutralizing antibody that is distinct from neutralization. For example, activity against infected cells, as well as (or in some cases instead of) activity against free virions, might be required to provide protection. This would be consistent with observations that the antibody concentrations that are required for activity against infected cells — for example, by blocking cell–cell transmission — are typically considerably higher than those that are required for the neutralization of free virus particles.

Animal models provide direct evidence that mechanisms other than neutralization can be important for protection by neutralizing antibodies. In some cases, protection is found to be as effective with $F(ab)_2$ fragments — which lack the Fc domain and, therefore, are unable to trigger effector functions — as with the corresponding whole IgG molecule. However, in other cases, $F(ab)_2$ fragments that are as effective as whole IgG molecules at neutralization *in vitro* are ineffective at protection *in vivo*. For yellow fever virus[39], it has been shown that neutralizing mouse IgG1 antibodies (which are poor activators of effector functions) are ineffective at protection, whereas IgG2a molecules (which are good activators) of the same specificity are effective. In many examples in mouse models, protection requires the Fc part of IgG, but is independent of complement. This implies that, in these cases, protection by neutralizing antibodies probably requires activity against infected cells and involves ADCC or phagocytosis.

There are many examples of protective activity shown by non-neutralizing antibodies in passive-transfer studies in animal models. This activity seems to be directed against infected cells and, generally, it seems to be somewhat less potent than the activity of neutralizing antibodies. For example, several cases have been reported in which neutralizing antibodies are protective against higher challenge doses or more-pathogenic viruses than are non-neutralizing antibodies. In many cases, protection mediated by non-neutralizing antibodies is shown to depend crucially on the Fc part of the antibody molecule and to occur in complement-deficient mice, which indicates that ADCC (or phagocytosis) might be crucial for clearing antibody-complexed infected cells. Protection mediated by non-neutralizing antibodies is restricted mostly to protection against enveloped viruses.

The passive transfer of antibodies to humans has been shown to provide protection against disease caused by several viruses, including hepatitis B, hepatitis A, measles, polio and RSV[40]. Indeed, a neutralizing anti-RSV monoclonal HUMANIZED ANTIBODY is in clinical use to protect at-risk infants[41]. In many of these cases, it is unlikely that the titres of passively transferred neutralizing antibodies reach the levels that are necessary for sterile protection (although this has not been studied widely). Rather, it would seem that neutralizing antibody sufficiently blunts the infection to allow the development of other protective mechanisms — presumably, $CD8^+$ T cells, active antibody responses and innate immunity (see below). The protection of young infants by maternal neutralizing antibodies probably falls into this category. Maternal antibodies "attenuate infection during the initial months of life, thereby creating optimal conditions for the natural immunization of the child as a result of infection"[3]. In some cases — for example, infection with rabies virus — the passive transfer of antibody has been shown to protect against disease after exposure, when some degree of infection is established. However, once infection is fully established, then reports of the beneficial effects of passive antibody transfer are rare.

Many important human viral pathogens gain entry to the host through mucosal surfaces. Passive-transfer studies show that antibodies that are present in the mucosal compartments at the time of exposure can protect against viral challenge[26,42]. Both mucosal secretory IgA (sIgA) and systemic IgG have been shown to be effective. Remarkably, non-neutralizing IgA can protect against rotavirus challenge in mice by an intracellular neutralization mechanism[43].

The antiviral activity of T cells

Passive-transfer experiments are useful to elucidate the protective activities of antibodies. However, they might not truly mimic most vaccine situations, because both the cellular immune response and a secondary B-cell response are absent. Before considering the role of antibodies in vaccine protection, it is worth briefly reviewing the antiviral activity of T cells. $CD8^+$ T cells can act against viruses through the specific recognition by their T-cell receptors (TCRs) of viral peptides bound to MHC class I molecules on the surface of the infected cells. The antiviral activity is manifested in target-cell killing, the induction of apoptosis in target cells and the release of antiviral cytokines that can clear viral infection from target cells[2]. Abundant evidence

Glossary

AGAMMAGLOBULINAEMIC
A person who has an inherited disorder that is characterized by very low levels of immunoglobulins.

DNA-SHUFFLED ENVELOPE LIBRARIES
Libraries of envelope molecules that are produced by *in vitro* homologous recombination of random fragments of envelope genes generated from pools of parental envelope genes.

HUMANIZED ANTIBODY
An antibody in which protein engineering is used to reduce the amount of 'foreign' protein sequence by swapping rodent antibody constant regions and the variable-domain framework regions with sequences that are found in human antibodies.

ORIGINAL ANTIGENIC SIN
A phenomenon in which the antibody response that is elicted in an individual after secondary viral infection reacts more strongly to the viral variant that originally infected the individual. Can also be shown for closely related antigens of non-viral origin.

SUBUNIT VACCINES
Vaccines that contain only a small part of the pathogen, such as the protein that forms the coat surrounding the nucleic acid of a virus. Usually produced by genetic engineering.

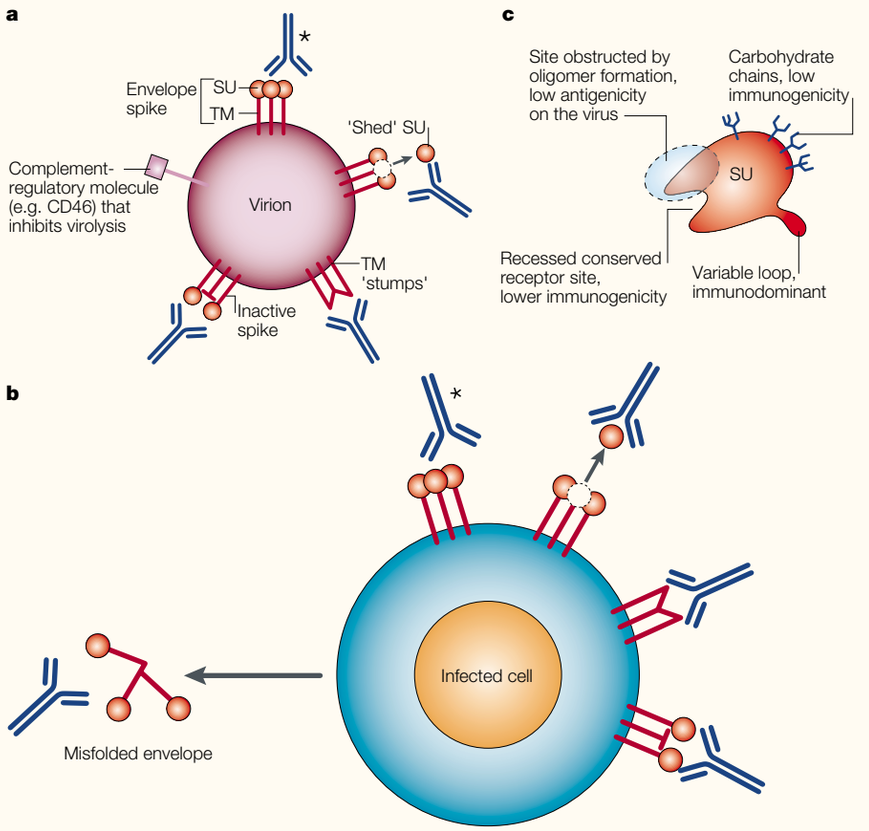

Figure 2 | **Viral evasion of antibody responses.** The diagrams are based on features that have been described for HIV-1, but many of these features are also shown by other enveloped viruses. **a** | The virion. Neutralizing antibody (*) binds to native envelope spikes, shown here as a homotrimer of heterodimers formed by the transmembrane protein (TM) and the surface glycoprotein (SU). Some envelopes — for example, of HIV-1 and Ebola virus — can shed SU, which might serve as a 'decoy' if antibody responses to shed SU have a reduced affinity for SU on the envelope spike. So, by the mechanism of ORIGINAL ANTIGENIC SIN[82–85], immunization with antigen 1 (here, shed SU) can establish a population of memory B cells such that subsequent challenge with related antigen 2 (here, SU in the envelope spike) stimulates a response of high affinity for antigen 1, but more moderate affinity for antigen 2. Complement-regulatory molecules, such as CD46, that are incorporated in the membranes of some enveloped viruses can inhibit virolysis. Partially disassembled or misfolded envelope spikes can be potent immunogens. If the antibodies that are elicited have a reduced affinity for correctly folded envelope spikes then, as for shed SU, these molecules could act as decoys to generate a suboptimal response to native envelope spikes. However, virion binding of these non-neutralizing antibodies could also, in principle, facilitate complement-mediated virolysis and phagocytosis. **b** | Infected cells. Similar envelope molecules to those that have been described for the virion are shown. In addition, infected cells can release misfolded or incompletely assembled envelope complexes that might function as decoys. **c** | Surface glycoprotein. An immunodominant, but variable, loop favours the induction of strain-specific rather than strain-cross-neutralizing antibodies, as for HIV-1 and influenza virus. Carbohydrate chains and oligomer formation conceal large parts of the protein surface, which reduces antigenicity and immunogenicity. A recessed receptor site might also reduce antigenicity and immunogenicity.

indicates the power of specific CD8$^+$ T-cell responses in controlling viral infection[2,44–48]. The activity of specific CD8$^+$ T cells is probably related to the functional avidity of their TCR for the particular peptide–MHC complex[49,50]. Specific antiviral CD4$^+$ T cells[46,51] can also have direct antiviral activity. However, their most important role seems to be providing help to CD8$^+$ T cells and B cells[52].

CD8$^+$ T cells, CD4$^+$ T cells and B cells have non-overlapping functions, and a series of studies has provided an elegant demonstration that resistance to Friend murine leukaemia virus (FMLV) in mice requires all of these functions[53]. Most convincingly, the adoptive transfer of immune spleen cells to naive animals shows that complete protection is only achieved when CD8$^+$ T cells, CD4$^+$ T cells and B cells are transferred[54,55]. Any combination of two cell types is insufficient to provide protective immunity.

Antibodies in vaccine protection

The level of neutralizing antibody that is induced correlates with the degree of protection against disease for several viral vaccines[2]. This does not necessarily mean that neutralizing antibodies are the agent of protection. In principle, they could simply be 'markers' of exposure to viral antigens as are, for example, antibodies to internal viral proteins. However, the protective activities that are described in passive-transfer studies indicate that neutralizing antibodies are unlikely simply to be markers and are more likely to be actively involved in resisting infection. But, vaccine-induced, antiviral, serum neutralizing-antibody titres might not reach the levels that have been described in passive-transfer studies to provide sterile protection in animal models. Such levels are even less likely to be maintained for many years after vaccination. Therefore, it is unlikely that neutralizing antibodies that are present in the serum of a vaccinated individual at the time of virus challenge are solely responsible for protection. One would predict that some virus-infected cells will escape elimination by antibody. How, then, is this infection likely to be contained?

CD8$^+$ T, memory B and plasma cells. The obvious candidate is viral-specific CD8$^+$ T cells, and there is ample evidence for the ability of these cells to control viral replication, as described above. Virus-specific CD8$^+$ effector T cells could already be present at challenge, be recruited from the vaccine-induced CD8$^+$ T-cell memory pool or be induced *de novo*. Another possibility is that increased antibody concentration (as a result of the stimulation of memory B cells by viral antigen) contributes to protection. So far, we have focused on pre-existing antibody as the most powerful first line of defence against viral challenge. This antibody can be maintained at relatively high levels for many years, probably produced by long-lived plasma cells[56,57], although this is not universally accepted[58]. In a sense, the most crucial part of antibody 'memory' might be equated with the long life of these plasma cells. However, the second antibody memory component, memory B cells, might also be crucial for vaccine-mediated protection in some cases. Equally, the contact of memory B cells with viral antigen might be important to boost plasma-cell numbers and serum-antibody concentrations for the next encounter with the virus. The blunting, rather than ablation, of infection will facilitate this boost by increasing the amount of antigen that is available. Finally, other mechanisms, particularly innate immunity, might contribute to containing infection.

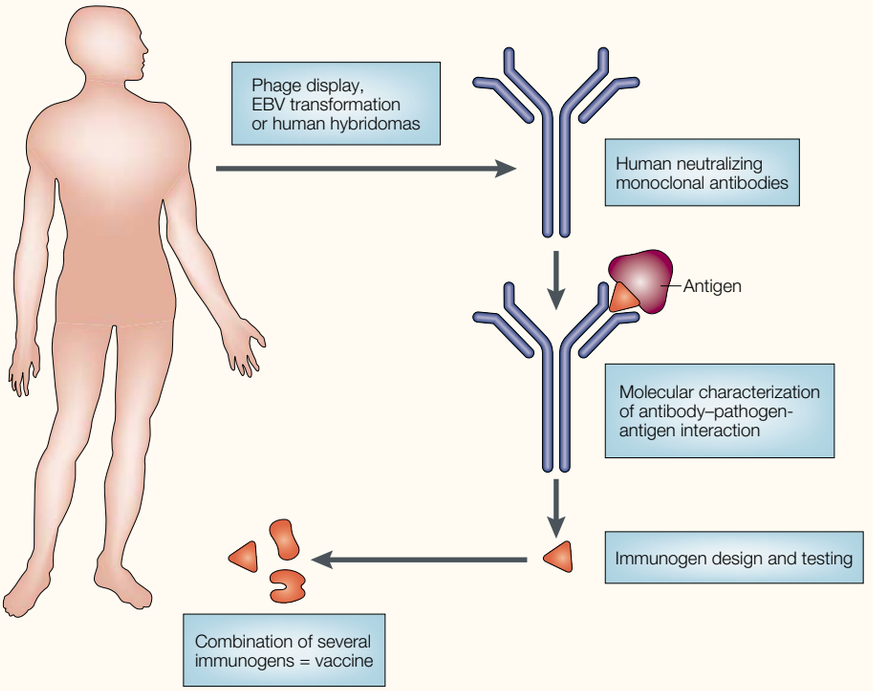

Figure 3 | **Reverse vaccinology.** Classical vaccine antigens, such as attenuated or killed virus preparations or subunit proteins, might fail to elicit significant protective antibody responses. If, however, monoclonal antibodies that are shown to mediate protection can be isolated from cases of natural infection (or immunization, for example of transgenic mice that express human antibodies), then these antibodies might allow the generation of immunogens that, when introduced as vaccines, elicit closely related protective antibodies. EBV, Epstein–Barr virus.

The relative importance of these mechanisms in most cases of vaccine-mediated protection is not known.

Protection and antibody-mediated blunting. From the antibody standpoint, I suggest that vaccine-mediated protection can be characterized according to the degree of blunting of infection by neutralizing antibody. The most complete blunting of infection corresponds to sterile immunity, which might be achieved in certain cases. For many vaccines, and especially as neutralizing-antibody titres decrease with time after vaccination, the blunting of infection is likely to be incomplete and other mechanisms of antiviral action must come into play. Non-replicating vaccines — such as killed poliovirus, hepatitis B, hepatitis A, rabies and influenza vaccines — elicit neutralizing antibody, but weak $CD8^+$ T-cell responses. Therefore, the control of residual infection is probably achieved by $CD8^+$ T cells that are induced *de novo* or antibody from activated memory B cells, or perhaps by innate immunity. For replicating vaccines — such as those against measles, mumps, rubella and varicella zoster viruses — the blunting of infection by neutralizing antibodies is also likely to be important, because the passive transfer of immunoglobulins seems to offer protection against disease[59–63]. These vaccines will also generate memory $CD8^+$ T cells that can be activated to effector $CD8^+$ T cells by contact with viral peptide. One might speculate that as the ability to blunt an infection declines after vaccination, the ability to recruit effectors ($CD8^+$ T cells and antibodies) rapidly from the memory compartment will become more important. Blunting will tend to be inefficient at mucosal surfaces, where the levels of vaccine-induced antibodies decline much more rapidly than in the serum[56]. A further point to note about replicating vaccines is that they might induce an antibody response to non-structural proteins that are expressed on infected cells, but not on virions. This might be crucial; for example, protection against tick-borne encephalitis virus (TBEV) has been correlated with such an antibody response[64].

For some viruses, blunting by neutralizing antibody alone, short of sterile protection, might always be insufficient. The studies on FMLV that are described above indicate that a vaccine that is unable to mobilize $CD8^+$ and $CD4^+$ effector T cells rapidly would be unable to prevent the establishment of a persistent infection of this virus.

Are antibodies necessary for protection? Is antibody-mediated blunting of infection truly indispensable for vaccine-induced antiviral protection? The observation that some individuals who have antibody deficiencies do not suffer from an increased incidence of certain viral diseases or increased disease severity indicates that this might not be the case. For infection with measles virus, it is reported that AGAMMAGLOBULINAEMIC children have a normal disease course and are subsequently immune to infection[65]. However, we have recently challenged the view that patients who are fully antibody deficient have been satisfactorily studied[66]. Patients who were diagnosed with antibody deficiency were treated from relatively early times with immunoglobulin-replacement therapy and, as this therapy improved, viral diseases diminished. Furthermore, a vaccine should seek to protect a wide spectrum of different individuals who are exposed to the virus in different circumstances, but the experiments in agammaglobulinaemic individuals have only been carried out on a relatively small scale. Nevertheless, although the situation in humans is debatable, it is clear from studies in several animal models that vaccines that induce only $CD8^+$ T cells, and no virus-specific antibodies, can protect against subsequent challenge with a viral pathogen. This was first shown more than a decade ago in the LCMV model[67], and it has been confirmed in other models, such as influenza virus[68] and RSV[69]. A cytotoxic T lymphocyte (CTL) peptide-based vaccine did initially protect four out of eight sheep against challenge with bovine leukaemia virus (BLV)[70], but on long-term follow-up, all but one of the animals seroconverted and progressed to disease[71] (A. Suhrbier, personal communication). Vaccines that elicit only a CTL response have been shown to mediate protection against disease after SHIV challenge[72], but not, so far, against SIV challenge[73]. Overall, therefore, vaccines that elicit only $CD8^+$ T-cell responses can confer sufficient immunity to offer some protection against subsequent challenge in some cases. However, in many cases, the recipients of these 'CTL vaccines' develop marked symptoms before recovery, which is consistent with the idea that $CD8^+$ T cells cannot prevent infection, but instead limit viral replication and dissemination (J. L. Whitton, personal communication). So, a vaccine formulation that induces only $CD8^+$ T-cell immunity is unlikely to provide the high degree of protection across a large population that we have come to associate with the term 'vaccine'.

Box 1 | **Understanding antibody-mediated vaccine protection**

- **How often is sterile protection achieved?**
- **How important is B-cell memory compared with pre-existing antibody in resisting infection?**
- **How important are effector functions in antibody-mediated protective activity?**
- **How important are antibody activities against infected cells compared with activities against free virus?**
- **How important are activities mediated by non-neutralizing antibodies?**
- **How important are co-operativity and synergism in antibody mixtures?**
- **How important are mucosal antibodies?**

Eliciting protective antibodies

The ease with which neutralizing antibodies can be elicited varies widely. It has been argued convincingly that repetitive viral surface antigens elicit the most potent antibody responses to virions[74,75]. For example, the densely packed, highly organized glycoprotein of vesicular stomatitis virus (VSV-G) induces a strong neutralizing antibody response that is independent of T-cell help. By contrast, when the soluble form of VSV-G is used as an immunogen, it fails to induce a neutralizing response. Therefore, a native form of VSV (an attenuated or killed virus) should make a good vaccine from an antibody perspective. Poliovirus is similar to VSV in that it induces a potent neutralizing antibody response. For such viruses, there is, presumably, no evolutionary pressure against a strong neutralizing antibody response. This would be the case, for example, for a virus that is transmitted from an infected to an uninfected individual before the development of a neutralizing antibody response that could interfere with transmission. Similarly, to avoid evolutionary pressure from the neutralizing antibody response, the virus should not depend on being able to re-infect the same individual to prosper. For some viruses that have relatively rigid and organized surfaces, such as influenza virus, it is argued[74] that there is an evolutionary pressure against a neutralizing response and that this leads to the selection of serotypes (viral variants) that escape binding by neutralizing antibodies. For this strategy to be effective, the neutralizing antibody response must be focused on an immunodominant epitope of the virion surface that can tolerate mutations without substantially affecting virion function. Structurally, this is generally achieved by surface loop regions that are highly accessible, immunogenic and potentially variable (FIG. 2). In this case, vaccination often requires a new immunogen for each serotype that is described.

Many viruses — for example, paramyxoviruses, poxviruses and herpesviruses — have less organized, more 'fluid' surfaces, and it is proposed that this is a strategy to avoid antibody responses[74]. Such viruses have often evolved many other features to avoid functional antibody responses. FIGURE 2 illustrates the targets for functional and non-functional antibodies on virions, infected cells and a typical envelope spike structure. The envelope spikes of some viruses have developed several features to minimize immunogenicity. One of these features is the burial of monomer protein surface in the oligomeric arrangement that forms the spike structure. It seems that, for some viruses (notably HIV-1), high titres of antibodies are found that are specific for the monomeric forms of envelope proteins, but that titres against oligomeric proteins are low. As oligomer binding is associated with neutralizing activity, this indicates that there are low neutralizing-antibody titres[76–78]. Monomeric envelope proteins might be produced as 'viral debris'[78–80]. A corollary of these observations is that monomeric envelope proteins might be poor vaccine candidates in some cases.

For viruses such as HIV-1 that have evolved many mechanisms to avoid neutralizing-antibody responses — particularly those that neutralize many different isolates — classical vaccination strategies, such as the use of attenuated or killed viruses, might be ineffective. If an envelope protein has been selected to minimize neutralizing-antibody responses, then simply mimicking the envelope might not be a useful vaccine approach[81]. For HIV-1, although cross-neutralizing antibody responses are weak, they do exist, and a panel of neutralizing human monoclonal antibodies has been isolated. We suggest that one route to a vaccine might be to produce immunogens based on an exploration of the interaction of these antibodies with envelope proteins. This technique is known as 'reverse vaccinology' (FIG. 3), because the central concept is to generate vaccines from antibodies, rather than the usual task of generating antibodies from a vaccine. Practically, the immunogens might be designed from our knowledge of complexes of antibody and envelope protein or, alternatively, by antibody-mediated selection of molecules from, for example, peptide libraries or DNA-SHUFFLED ENVELOPE LIBRARIES.

Concluding remarks

Viruses are an enormously heterogeneous group of pathogens, and individual human exposures and immune responses vary widely, so that generalizations with respect to immune protection are to be treated with great caution. Nevertheless, it is probable that most exposures of naive individuals to many, but by no means all, viruses are resolved successfully without long-term adverse effects. Typically, resolution does not require the action of neutralizing antibody, because such antibody often appears after symptoms have abated. Cellular and innate immunity are probably crucial to the resolution of infection. However, on re-exposure of the individual, neutralizing antibodies can act very rapidly to blunt infection, so that it can be contained by cellular and innate immunity without symptoms of disease. Vaccination is probably successful in some cases quite simply because it provides this antibody-mediated blunting effect. However, for some individuals, exposure conditions or viruses, antibody-mediated blunting is insufficient and, then, the best hope for containment lies in the additional rapid deployment of specific $CD8^+$ T cells. If HIV-1 is ever to be controlled by vaccination, it will probably require a vaccine that leads to efficient antibody-mediated blunting and a rapid, potent $CD8^+$ T-cell response.

Given the importance of antibodies for vaccine protection, it is surprising how little we understand about how antibodies fulfil this function in humans. The term 'neutralizing antibodies' might lull us into a belief, which has not been shown formally, that these antibodies operate *in vivo* solely by the mechanism(s) of neutralization that have been observed *in vitro*. As I have discussed, neutralizing antibodies can operate by several mechanisms. BOX 1 summarizes some of the questions that need to be answered about vaccine-induced antibodies. Many of these questions probably need to be answered separately for each individual virus that is studied. Answering these questions will facilitate a more rational approach to vaccine design and will lead ultimately to more-effective vaccines.

Dennis R. Burton is at the Departments of Immunology and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA.
e-mail: burton@scripps.edu

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