Stanley A. Plotkin, Section Editor

Complex Correlates of Protection After Vaccination

Stanley A. Plotkin

Emeritus Professor of Pediatrics, University of Pennsylvania, Philadelphia, PA

In several prior articles I have attempted to analyze and simplify the subject of immunological functions induced by vaccination that correlate with protection against later exposure to pathogens. Other authors have also written on the subject, and recently we jointly proposed terminology to bring some semantic clarity to the field. The generalization that vaccine-induced antibodies prevent acquisition whereas cellular immune functions clear infection still holds true, but that simple distinction becomes blurred in many instances. Specific antibody and cellular responses are multiple and redundant, so that vaccines for some pathogens protect through more than 1 immune function. Thus, this article aims in the direction opposite to simplicity to depict the complexity of correlates, or rather the complexity of mechanistic immune functions that contribute to protection. Nonmechanistic correlates that are practically useful but not truly protective will be mentioned in passing.

Keywords. correlates; pertussis; cytomegalovirus; HIV; ebola.

"*Everything should be made as simple as possible, but not simpler.*" —Albert Einstein

MECHANISTIC IMMUNE RESPONSES

The flowering of immunology has resulted in the identification of many different B- and T-cell functions [1–3]. Antibody differs not only in quantity, but in avidity and in the specificities of the epitopes on proteins or polysaccharides of the microbial pathogen that is targeted. Memory, both effector and central, is key to the success of vaccines in practical use. In general, central memory is most useful in diseases with long incubation periods, but effector memory, the persistence of cells producing antibody, is critical to protection against most infections. The identification of T-cell subsets has introduced new complexities:

Whereas the T-helper cell (Th) 1/Th2 distinction is still important, we now have Tfh (follicular helper T cells), Th17, and Tregs (regulatory T cells). Tfh cells are important in expansion of B cells, Th17 cells act to prevent mucosal carriage of microbes, and Tregs modulate immune responses. In addition, the roles of both CD8+ and CD4+ T cells have long been recognized as important in killing infected cells. Thus, a large variety of immune cells and functions are involved in controlling infections and any assignment of one as the mechanistic immune functions that contribute to protection (mCoP) must recognize that others may be involved as supplements or co-correlates of protection. Elsewhere we have reviewed the correlates of protection for all licensed vaccines [4], whereas here we cite a few selected examples of licensed vaccines and interesting examples of vaccines in development.

QUALITATIVE FACTORS THAT INFLUENCE MECHANISTIC CORRELATES

As has been pointed out previously [4–7], protection must be defined as against what: mucosal infection without spread, invasion, symptomatic infection, severe disease, or other clinical manifestation? The

timing of the test of immunity, whether taken at the peak of response after vaccination or just before exposure to infection, may give different results. In addition, both pathogen and host factors must be taken into consideration in defining a correlate. Infectious dose is the most important pathogen factor, with large doses requiring larger host immune responses to protect. Host factors that influence correlates include age; genetic susceptibility, with major histocompatibility complex (MHC) types being the most influential; nutrition, as it influences host responses; prior sensitization to organisms related antigenically to the pathogen; and perhaps ethnic differences.

The best example of the quantitative variability of an mCoP is invasive pneumococcal disease. Protective opsonophagocytic antibody levels differ among serotypes [8, 9]. In addition, although pneumococcal conjugate vaccine is efficacious everywhere in the world, the level of antibody necessary to prevent invasive pneumococcal disease is higher in Africa than in the United States [9–12]. Also, protection against mucosal infections such as acute otitis media clearly requires more serum immunoglobulin G (IgG) antibody, presumably because diffusion from serum is required if local secretory immunoglobulin A (IgA) is absent [13].

LICENSED VACCINES

Influenza
The multiple correlates of protection that have been proposed for influenza vaccines provide a perfect example of the complexity of the subject [14]. Clearly, serum antibody is an important mCoP, as measured either by hemagglutination inhibition (HAI) or microneutralization and plays a role in clearance as well as prevention of acquisition [15, 16]. Although an HAI titer of 1:40 has been taken by regulatory authorities as the level that will protect most people, there is disagreement as to whether that number is correct, and in any case protection seems to be a continuous function, with higher titers giving higher levels of protection [17]. In a study of a cell-culture produced vaccine, a titer of 1:15 was claimed to be adequate [18], although to this reader it appeared that 1:30 was a safer bet. On the other hand, in children it has been claimed that a titer of 1:110 is necessary [19]. In another study, some doubt was expressed about the value of serum antibody, but the data appeared to support the protective value of titers >1:32 or >1:64 [20]. Thus, although no serum antibody titer is completely protective in itself, nevertheless the 1:40 titer appears to be a reasonable statistical correlate for an efficacy of 50%–70% against clinical symptoms of infection.

However, influenza is not an invasive infection, so clearly in order to prevent viral replication, antibody must be present on the nasopharyngeal and pulmonary mucosa. It is well known that mucosal antibody is predominantly IgA in the nasopharynx, but in the lungs IgG antibody is important. In the case of the inactivated vaccine it is the IgG response that is critical, whereas the live attenuated vaccine does induce local IgA, and in a challenge study, protection was correlated both with serum and mucosal antibody [21, 22]. In mice, a live vaccine against the H5N1 virus was protective only if antibody was elicited in the lungs [23]. Th1 cell frequency correlated with serologic response to an H5N1 vaccine, but protection was not measured [24].

It is also well established that cytotoxic T-cell responses, both CD4$^+$ and CD8$^+$, are responsible for controlling and terminating influenza infection and may be a correlate of heterotypic protection [25–28]. In the elderly, this fact becomes even more important, as they mount neither high antibody responses nor strong cellular responses, which explains the low efficacy of influenza vaccines in older individuals relative to younger subjects [29, 30]. In particular, lower granzyme B activity of mononuclear cells in the blood of elderly vaccinees differentiates those who develop influenza despite vaccination from those who do not [31]. Impaired innate immune responses in the elderly also contribute to lower adaptive responses [32]. The live influenza vaccine produces good cytotoxic T-lymphocyte responses, whereas the inactivated vaccine does not [33], and those responses may be responsible in part for the better efficacy of live over killed vaccine in children [34], who do not have the immunological benefit of T-cell memory induced by prior influenza infection. An experimental vaccine relying on T-cell responses to the viral matrix protein 1 and nucleoprotein showed some reductive effect on symptoms and virus shedding [35]. Moreover, a recent study in humans showed a better correlation of cytotoxic CD4$^+$ T cells with control of replication than CD8$^+$ T cells, again indicating that multiple factors play a role in immunity to influenza [36].

In summary, although serum antibodies are the most useful mCoP for inactivated influenza vaccines containing viral hemagglutinin, mucosal antibodies clearly contribute to prevention of infection, and cytotoxic T lymphocytes are important for heterotypic protection and to reduce viral replication and resulting disease if infection is not prevented [37, 38]. Moreover, if new antigens such as neuraminidase, M2e, nucleoprotein, or others are added to the vaccine, new mCoP involving either humoral or cellular immune responses will become relevant [39, 40].

Pertussis
Much ink has been spilled in an effort to describe the correlates of protection against pertussis, and the cliché one often reads is that they do not exist. In my opinion, the opposite is true: Correlates exist but are multiple. In any case, an answer to the question has become urgent because of the resurgence

of pertussis in many countries, contemporaneous and possibly related to the switch from whole cell to acellular vaccines. It is essential to understand that pertussis is above all a toxic disease and that production of antitoxin by a vaccine is essential to protection. Low levels of pertussis antitoxin correlate with susceptibility to disease [41]. However, infection involves attachment to cells in the upper and lower respiratory tract, and antibodies that interfere with attachment can protect. Thus, filamentous hemagglutinin, pertactin, and fimbrial agglutinogens in varying combinations have all been included in acellular vaccines. Pertactin may be particularly important in the generation of opsonophagocytosis [42]. Studies during outbreaks have shown that high levels of antibodies to pertussis toxin, pertactin, and fimbrial agglutinogens protect singly and synergize: that is, having antibodies to any one gives some protection and better protection is given by antibodies to 2 or all 3 of the antigens [43–47]. Whole cell vaccines automatically contain these components, as well as others less well described, and additional toxic moieties aside from pertussis toxin. Whole cell vaccine also contains lipopolysaccharide and is therefore autoadjuvated [48]. Bactericidal activity may be important, and this function of antibody after vaccination may be inferior to that generated by natural infection [49, 50].

However, antibodies are not the whole story. Studies in mice at least suggest that Th1 cellular responses give longer-lasting protection, whether in themselves or as help to antibody persistence [51, 52]. In addition, a role of Th17 cells in prevention of carriage is suspected [53]. The cause of resurgent outbreaks of pertussis is unknown, but high on the list of suspects is waning antibody after acellular vaccines, perhaps because of a Th response that is Th2-directed rather than the Th1 type provided by whole cell vaccines [54–58].

Whether purely on the basis of antibodies or on some combination of antibodies and T cells, it is virtually certain that waning of immunity after vaccination is a major cause of the recent resurgence of pertussis [59]. New vaccines may be needed [60].

Poxviruses

The situation for vaccinia-induced protection has been well described, and is similar to that following natural smallpox. Serum antibody is the sine qua non for successful protection against infection after vaccination, and lasts a lifetime [61]. $CD4^+$ T-cell responses are also long-lasting, whereas $CD8^+$ T-cell responses fade with time [62–64]. Although $CD8^+$ T-cell responses are clearly important in preventing severe disease after exposure to poxviruses, they are insufficient in themselves to prevent infection but are complementary to antibody [65]. Thus, the mCoP for smallpox and vaccinia is serum antibody, as is confirmed by the role of passive antibody in treating complications of vaccinia, but cellular responses do contribute

importantly to control of viral replication [66]. In a murine model, rapid induction of $CD8^+$ T-cell perforin production gave better prevention of mortality than did antibody [67].

Rotavirus

The 2 orally administered live rotavirus vaccines have been tremendously effective in prevention of both infection and disease by the multiple serotypes of the virus, but correlates of protection have been elusive. For an enteric infection, it is tempting to believe that intestinal mucosal antibody is key to protection. In animal models, it has been possible to clearly implicate secretory IgA [68], but the difficulty in measuring intestinal IgA in humans has been problematic. The most commonly used measure of response to vaccination is serum IgA, and there is good statistical correlation with protection, but serum IgA is not a measure of secretory IgA and it is currently uncertain as to whether it is a mechanistic or nonmechanistic correlate of protection (M. Patel, personal communication, December 2012).

Beyond the questions about which intestinal immune response is the mCoP, the great controversy for rotavirus vaccine concerns the relative importance of homotypic immunity and heterotypic immunity. Type-specific antibodies are elicited by vaccination against the vp4 and vp7 proteins that are neutralizing antigens, but the fact that a monovalent vaccine is as effective as a pentavalent vaccine indicates that heterotypic immunity is substantial, although it should be noted that the monovalent vaccine bears the most common human P and G serotypes. In contrast, although infections with animal rotaviruses with different P and G serotypes induce a degree of immunity in humans, protection is improved by vaccine viruses bearing the human serotype proteins. To further complicate matters, viral antigens that induce nonneutralizing rather than neutralizing antibodies, including vp6 and the NSP4 nonstructural viral toxin, also generate protection in animal models. In addition, infants who have severe combined immunodeficiency may develop chronic rotavirus infection, suggesting a role for cellular immunity at the intestinal level [69]. The safest conclusions seem to be that an immune response in the intestine is protective but is not necessarily neutralizing in the classic sense and that there is an advantage to infection with rotaviruses carrying human serotype proteins [70–73].

VACCINES IN DEVELOPMENT

Cytomegalovirus

A large number of candidate vaccines are in development to prevent infection and resultant damage of fetuses by maternal acquisition of cytomegalovirus (CMV), as well as CMV disease in transplant patients [74, 75]. In the case of the latter,

both primary infections in seronegative solid organ transplant recipients and reactivation in seropositive hematopoietic stem cell transplant (HSCT) recipients cause serious disease. Recent clinical trials of the adjuvanted CMV glycoprotein B in young women and in solid organ transplant recipients have shown protection that can only be attributed to neutralizing antibodies, although it is possible that some function of antibodies other than neutralization is in play [76]. However, the picture is complicated by the fact that neutralizing antibodies that prevent entry into epithelial and endothelial cells, and thus have perhaps greater functional importance, are induced by a complex of 5 other proteins on the virus, one of which is glycoprotein H [77]. Antibodies to this pentameric complex are responsible for the majority of neutralization of CMV by sera from natural seropositive individuals and human immunoglobulin [78, 79], and there is some evidence for their importance in reducing maternal-fetal transmission [80]. Thus, with regard to antibodies, there may be 2 separate mCoP for a CMV vaccine.

The role of cellular immune function in CMV is also nuanced. It certainly appears that T cells, both CD8+ and CD4+, control viral load and that a vaccine for HSCT recipients should induce them [81, 82]. Passively administered CD8+ cells sensitized to CMV have been useful in those patients [83]. In addition, some data suggest that maternal CD4+ T cells play a role in prevention of transmission to the fetus [84]. However, it is unclear whether cellular immunity will be needed in a vaccine and what particular cellular response, if any, is an mCoP. Moreover, many proteins of the virus induce CD8+ cytotoxic T-cell responses, although judging from protection in vaccine studies, the immunodominant tegument protein pp65 seems the most important [81, 85]. The mCoP for a CMV vaccine is likely to be defined only by efficacy trials.

Ebolavirus

In view of the rarity of Ebola infections, it will be difficult to determine a correlate of protection in human studies with good power. However, the disease can be modeled most effectively in nonhuman primates and there the picture is reasonably clear: An antibody response correlates well with protection but is insufficient unless there is a concomitant cellular response. The key responses are directed against the viral glycoprotein. In nonhuman primate studies, an enzyme-linked immunosorbent assay titer of 1:3700 against the glycoprotein was associated with high-level protection [86]. Nevertheless, passive administration of the same antibody did not completely protect, so a cellular response appeared necessary. This was confirmed by the demonstration that an Ebola vaccine based on an adenovirus type 5 vector carrying the gene for the glycoprotein required a CD8+ T-cell response to be effective [87]. Yet in studies using other antibodies, protection was achieved by passive transfer [88, 89]. It may be that antibody is not neutralizing in vivo but is rather collaborating with T cells to give antibody-dependent cellular cytotoxicity. Other possible T-cell mechanisms include secreted cytokines, cytotoxicity, or simply help for antibody responses. However, it appears that antibody will provide a sufficient correlate of protection for the purpose of licensure of the most advanced candidate Ebola vaccines, even if it is a nonmechanistic correlate that is practically useful but not truly protective [90].

Human Immunodeficiency Virus

It is only with trepidation that one can venture to discuss correlates of protection against human immunodeficiency virus (HIV), inasmuch as none has been established after natural infection and only 1 vaccine trial has shown efficacy. Nevertheless, certain concepts are beginning to emerge. In nonhuman primate models, neutralizing antibodies directed against the V3 loop or gp41 have been shown to protect against acquisition of strains of HIV [91–94] and even to prevent superinfection [95]. This fits with classical notions of viral immunology. However, in addition, polyfunctional CD8+ T cells suppress viral load after challenge with simian immunodeficiency virus (SIV) [96–98], and also were shown to reduce replication of transmitted founder viruses in humans [99]. Remarkably, a rhesus cytomegalovirus vector carrying SIV genes was able to cause abortive infection in a significant percentage of monkeys challenged with SIV, which correlated with the induction of effector CD8+ T cells [100]. This result suggests that effector T cells are an mCoP for termination of acute infection. An even greater surprise came from a trial of a canarypox-HIV envelope vector prime followed by an envelope gp 120 protein boost that showed 31% efficacy against infection [101]. Protection correlated with the induction of nonneutralizing antibody directed against the V2 loop of the envelope [102]. A sieve analysis comparing isolates from placebo and vaccine recipients supported the importance of V2 antibodies [103]. An indication that V2 antibodies may be important with other regimens comes from recent data showing their induction by vaccination with adenovirus vectors that protect monkeys against SIV [104]. But the situation is even more complex: Antibody-dependent cellular cytotoxic (ADCC) antibodies synergize with V2 antibodies to kill HIV infected CD4+ T cells, whereas serum monomeric IgA blocks the action of ADCC antibodies and diminishes protection [105]. Thus, many different antibody functions must be measured in vaccine studies [106, 107], and a future HIV vaccine may depend for success on several mCoPs, including specific cellular immunity [108, 109].

Malaria

At long last, a vaccine against malaria has reached the stage of being a serious candidate for licensure. Of the 4 stages of the

parasites in humans—sporozoites, liver stage, merozoites, and gametes—the first 2 have seemed the best targets for prevention of infection, and human challenge with sporozoites has been well used to identify possible vaccine antigens and to study correlates of protection. The most advanced malaria vaccine in clinical trial is the RTS,S/AS01 vaccine, which contains a large portion of the circumsporozoite antigen (CSP), expressed on a hepatitis B surface antigen particle, and which has given modest protection [110]. Initial studies showed that children with higher anti-CSP titers were more likely to be protected against infection, although once infected their malarial illness was not different [111]. However, it was apparent during development that antibody was not the only story and inclusion of an adjuvant capable of driving both antibodies and CD4$^+$ T cells was critical to vaccine efficacy [112, 113]. In one of the first examples of the utility of systems biology, it was shown that genes associated with immunoproteosome processing of peptides for presentation to MHC groups were upregulated in protected vaccinees [114]. Study of T cells after vaccination showed that effector and central memory CD4$^+$ T cells secreting CD40L and interleukin 2 together with some interferon-γ and tumor necrosis factor–α were correlated with protection against clinical malaria [115–117]. Thus, it appears that protection by RTS,S vaccine is mediated both by CSP antibody and specific functional CD4$^+$ T cells. Apparently these 2 functions synergize with each other. Nevertheless, it was difficult to derive an quantitative threshold of absolute protection, and these correlates apply only to the circumsporozoite vaccine. In contrast, CD8$^+$ cells appear to be more important in protection produced by liver-stage antigens like TRAP and merozoite antigen MSP1 [118, 119]. Ultimately, a malaria vaccine containing components from multiple stages of the parasites will certainly have complex mCoP.

Reflections

The examples of complexity of correlates of protection given above do not gainsay the fact, emphasized in previous articles [4–7, 123], that antibodies are often the sole mechanistic correlates of protection. Infections in which pathogens must pass through the bloodstream in extracellular form, infections prevented by transudated antibody on the mucosa, and infections in which toxin production is the pathogenetic mechanism illustrate this point [120–122]. Nevertheless, many infections are more complex, and therefore mCoP will be multiple and will depend on the antigen(s) chosen for the vaccine, as well as age and other population factors. As vaccinology progresses, no doubt certain innate immune responses that facilitate B- and T-cell responses will be identified as mechanistic correlates [96, 124, 125]. Aside from the theoretical value of such discoveries, knowledge of mCoP will lead to faster development of more effective vaccines.

Notes

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