Development, Acceptance, and Use of Immunologic Correlates of Protection in Monitoring the Effectiveness of Combination Vaccines

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The establishment of immunologic correlates of protection for all vaccine antigens is a worthwhile goal. It allows new vaccines to be licensed on the basis of attainment of defined immunologic benchmarks, without the need for large-scale efficacy trials for each new product. This is particularly important for the evaluation of new combination products. Efficacy trials of each new mixture would be unethical because routinely recommended vaccines would be denied children in the control group. The establishment of immunologic correlates of protection should be a defined goal of every efficacy trial. Additional ways to evaluate the immune responses—such as cell-mediated immunity, mucosal immunity, memory responses, and antibody avidity—should also be studied. Finally, ongoing surveillance efforts are also needed, to monitor the impact of new and combined vaccines on disease rates.

During the evaluation of new vaccine candidates, initial studies focus on monitoring the safety and immunogenicity in a small number of normal, healthy adults and, in each successive study, progressively younger children [1]. Immunogenicity is usually measured by the detection of levels of humoral antibody to the target vaccine antigens. After these early studies demonstrate safety and immunogenicity, larger numbers of the target population are enrolled in studies of vaccine efficacy. The purpose of efficacy trials is to determine whether the vaccine provides protection against culture- or serologically confirmed disease. In many of the efficacy trials, humoral immune responses are measured in all participants or in a subset of the enrollees, with the express purpose of correlating antibody levels with vaccine efficacy. When specific immune responses to vaccine antigens are directly correlated with disease prevention (vaccine efficacy), a correlate of protection can be defined. Generally this correlate of protection is a defined level of antibody. However, this correlate may or may not represent the true mechanism of protection. For example, the level of humoral response after vaccination may correlate with prevention of disease, but the actual method of conferring protection may be cellular immunity. Because the humoral and cellular responses correlate well with each other, they both correlate with disease protection. The fact that antibodies are not a true surrogate of protection is often unrecognized by vaccinologists [2].

A thorough understanding of disease pathogenesis and mechanisms of protection is fundamental to establishing meaningful correlates of protection. The mechanisms of immunity involved in establishing protection against encapsulated extracellular bacterial pathogens are quite different from those involved in preventing, limiting, and clearing intracellular infec-
tions. These observations are summarized in Table 1. As is shown, antibody serves a major role in preventing, limiting, and clearing extracellular bacterial pathogens. Antibody production is aided by Th helper cells termed “Th1 cells” (CD4+Th1); these cells secrete cytokines, such as IL-4, and promote B cell development and antibody protection. Little role exists for T helper cells termed “Th2 cells” (CD4+Th2) and for T suppressor cell populations (CD8+Tc1). In contrast, for infections caused by intracellular pathogens, antibody has a major role in preventing infection, less of a role in limiting it, and little or no role in clearing it. Although CD4+Th1 cells are somewhat involved in limiting and clearing infection, CD8+Tc1 cells play a much larger role. As mentioned above, because humoral immune responses to vaccine antigens are the standard measures of immunogenicity and mucosal and cell-mediated responses are assessed less commonly, for most vaccines, humoral antibody levels are the only available measures to correlate with protection.

Methods to assess antibody levels as correlates of protection are complicated by several factors. First, humoral antibodies can develop in the absence of vaccination, because of natural exposure to cross-reactive bacteria. This is demonstrated in figure 1 (from the original work of Wright and Fothergill published >4 decades ago [3]). The dashed line signifies the incidence of cases of invasive Haemophilus influenzae type b (Hib) disease from infancy to adult life. As can be seen from the figure, the highest incidence of disease occurs in young children 6–12 months of age; after 2 years of life, invasive disease is rarely seen. The solid line reflects the bactericidal power of the blood measured by the ability of the serum to opsonize and kill Hib organisms in the presence of phagocytes. Bactericidal antibodies were noted at birth but fell to undetectable levels at 6 months of age, reflecting the catabolism of passively transmitted maternal antibody. Naturally acquired bactericidal antibody levels were detected as early as 3 years and peaked by 8 years of age. The inverse correlation between the incidence of invasive Hib disease and the presence of “bactericidal power in the blood” was striking and provided important data to predict the level of naturally acquired antibody needed for prevention of invasive disease.

Another important way to quantitatively determine the level of antibody associated with disease prevention is through the use of passive antibody administration. In patients with immunoglobulin deficiencies, the administration of defined amounts of specific antibody has been correlated with protection against disease. The delivery of antibody directed against polysaccharide bacterial capsular material was evaluated in Native American infants [4]. A human hyperimmune globulin termed “bacterial polysaccharide immune globulin” (BPIG) was prepared from plasma donors immunized with Hib, pneumococcal, and meningococcal vaccines. At a dose of 0.5 mL/kg, BPIG increased levels of antibody to Hib by >0.15 µg/mL within 4–6 h and by 2–4 µg/mL at 72 h. Thereafter, antibody declined, with a mean half-life of 27 days. BPIG treatment of Apache infants did not impair their active antibody responses to concurrently administered diphtheria-tetanus-pertussis or Hib oligosaccharide-diphtheria CRM197 conjugate vaccines. In high-risk Apache infants, BPIG provided at 2, 6, and 10 months of age provided significant protection from invasive Hib infection during infancy and provided a general correlate of antibody that was associated with protection.

As mentioned above, postimmunization antibody levels can be determined in participants of efficacy trials and levels can be associated with disease prevention. An excellent example of such a correlate was recently proposed as a result of the Northern Kaiser Pneumococcal Conjugate Vaccine Trial [5, 6]. In figure 2, antibody concentrations (in micrograms per milliliter) are shown for the infants receiving either the control vaccine or the 7-valent pneumococcal conjugate vaccine. Because few of the control and nearly all of the 7-valent vaccine recipients achieved levels of antibody of 0.15–0.50 µg/mL, these levels of antibody likely bracket the immunologic “correlates of protection.” Also, there has been some suggestion that the level of antibody needed to achieve protection may differ among the serotypes.

Several caveats are needed before one can accept antibody-based correlates of protection. First, antibody levels could increase after vaccination and could correlate with protection but might not be the primary immune mechanism associated with prevention of disease, as mentioned above. Second, considerable controversy surrounds some of the previously established correlates of protection [7–9]. Finally, additional methods need to be pursued in analyzing immune responses. These include measurement of memory responses, identification of determinants of antibody avidity, characterization of the mucosal immune responses at the area where the pathogen is introduced, and, finally, characterization of cell-mediated immune (CMI)

<table>
<thead>
<tr>
<th>Role in limiting disease</th>
<th>Antibody</th>
<th>CD4+Th2</th>
<th>CD4+Th1</th>
<th>CD8+Tc1</th>
</tr>
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<tbody>
<tr>
<td>Prevent infection</td>
<td>+++</td>
<td>−−−−</td>
<td>−−−−</td>
<td>−−−−</td>
</tr>
<tr>
<td>Limit infection</td>
<td>+++</td>
<td>−−−−</td>
<td>++</td>
<td>−−−−</td>
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<tr>
<td>Clear infection</td>
<td>+++</td>
<td>−−−−</td>
<td>−−−−</td>
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Intracellular pathogen

| Prevent infection         | +++      | −−−−    | −−−−    | −−−−    |
| Limit infection           | +++      | −−−−    | ++      | −−−−    |
| Clear infection           | −−−−     | −−−−    | ++      | −−−−    |

NOTE. ++++, Major role; −−−−, no role.
Figure 1. Incidence of cases of invasive *Haemophilus influenzae* type b (Hib) disease from infancy to adult life (dashed line). Bactericidal power of the blood was measured by the ability of the serum to opsonize and kill Hib organisms in the presence of phagocytes (solid line). From [3].

responses. One example of the use of memory responses to Hib conjugate vaccines was reported by Goldblatt et al. [10]. Geometric mean antibody titers to the polysaccharide of the Hib organism, PRP, were measured after a primary series of vaccinations at 2, 3, and 4 months of age and were found to be 1.23 μg/mL. When the immune response was measured at 15 months, the antibody level had declined to only 0.25 μg/mL. However, after administration of plain polysaccharide PRP at 15 months, the level of antibody increased to 7.86 μg/mL, reflecting a definite booster response because administration of plain polysaccharide in an unprimed toddler would have not stimulated such a vigorous response. The same group of investigators evaluated the avidity of the antibody after the boost in children with antibody levels of <1 μg/mL and in those with levels >1 μg/mL. Both groups of children had brisk responses to the PRP boost, but the group with the highest preboost titers had the highest antibody avidity [11].

Some caveats are necessary with these data. First, the mean age of boosting was 16 months and may not reflect responses seen during infancy. Second, the number of infants with antibody levels <0.15 μg/mL after the primary series was small, making it difficult to draw definitive conclusions about the lowest responders. Finally, short-term kinetic data, 24–48 h after boosting, were not provided to determine whether the memory response would be rapid enough to avoid invasive Hib disease. However, this is a good example of the use of antibody avidity to characterize immune responses.

CMI responses to pertussis antigens in infants primed with trivalent acellular pertussis vaccines have been demonstrated to persist longer than humoral responses [12]. Children were evaluated before the administration of a booster dose of acellular vaccine in infants previously primed with 3 doses of the same vaccine as infants. In these studies, conducted by Zepp et al. [12] in Germany, robust CMI responses were noted before the boost, although the levels of humoral immunity had declined remarkably. This suggests that persistent CMI can be detected in children before the booster dose.

How should correlates of protection be used in the evaluation of new combination vaccine products? First, correlates of protection can permit the licensure of new vaccines on the basis of immunogenicity studies that compare the performance of the new vaccines with vaccines of proven efficacy. If every new combination vaccine developed needed to be evaluated in efficacy trials, the cost of development of the products would be prohibitive. Also, it would be ethically difficult to enroll infants in an unvaccinated control group when a vaccine is recommended for use in all infants. However, the focus on immunogenicity results should be placed in a clinical context. Numerical differences may be statistically but not clinically significant. The identification of correlates of protection is suf-

Figure 2. Antibody concentrations in micrograms per milliliter for the infants receiving either the control vaccine or the 7-valent pneumococcal conjugate vaccine. Because few of the control and nearly all of the 7-valent vaccine recipients achieved levels of antibody of 0.15–0.50 μg/mL, these levels of antibody likely bracket the immunologic correlates of protection. From [8].
sufficiently important that it should be an explicit objective of all efficacy trials.

How can additional initiatives be supported to provide new and improved correlates of protection? Sufficient research funding should be provided to understand the pathogenesis of disease and mechanisms of protection for the vaccine-preventable diseases targeted. Correlates of protection should be sought routinely when evaluating vaccine efficacy. The newest immunologic techniques should be used to derive appropriate correlates of protection. Collaboration of vaccine evaluators with basic immunologists should provide translational information needed to better monitor meaningful immune responses. Surveillance should be conducted to detect disease rates over time after vaccines are routinely introduced. This would allow the detection of reduced efficacy of vaccine and would stimulate additional studies of the immune responses in children.

The recent resurgence of *H. influenzae* disease in the Alaskan population was detected by ongoing surveillance and highlights the importance of such methods in monitoring vaccine effectiveness [13]. Before vaccination, Alaska Natives experienced very high rates of invasive Hib disease and carriage. Vaccination with Hib conjugate vaccine polyribosylribitol phosphate *Neisseria meningitidis* outer-membrane protein began in 1991 and resulted in a sharp decline in cases. In 1996, after a different Hib conjugate vaccine, known as DTP-HbOC (which combines diphtheria-tetanus-whole cell pertussis vaccines with HbOC [Hib oligosaccharide CRM197]), came into use, cases of invasive Hib disease increased. The Hib experience in Alaska may indicate both the need for attainment of antibody levels early in the at-risk population and the need to achieve higher titers of antibody to prevent ongoing Hib transmission. Finally, cases of vaccine failure should be confirmed by culture and the immune factors leading to vaccine failure should be evaluated. Prospective multicenter trials should be in place to evaluate these vaccine failures, because no one center will likely have enough cases of vaccine failure to support such investigations.

In summary, the establishment of immunologic correlates of protection for all vaccine antigens is a worthwhile goal. It allows new vaccines to be licensed when defined immunologic benchmarks have been attained, without the need for large-scale efficacy trials for each new product. This is particularly important for the evaluation of new combination products. Efficacy trials of each new mixture would be unethical because it would require vaccines that are routinely recommended be denied children in the control group. The establishment of immunologic correlates of protection should be a goal of each efficacy trial. Some new combination vaccines, such as the acellular pertussis vaccines, have been licensed on the basis of comparative studies without clear immunologic correlates of protection. However, interpreting the significance of a decreased immunologic response to the new vaccine is this setting can be problematic. Innovative methods to evaluate the immune responses, such as cell-mediated immunity, mucosal immunity, memory responses, and antibody avidity, should also be evaluated. Finally, ongoing surveillance efforts are needed, to monitor the impact of new and combination vaccines on disease rates.

### References