Clinical Perspective

Use of vaccines in the evaluation of presumed immunodeficiency

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Clinical Problem

The assessment of the humoral immune response is a critical component in the evaluation of patients suspected of having a primary immunodeficiency disease (PIDD). In fact, more than 50% of the patients with immune deficiency have some impairment in the ability to make specific antibodies and/or have a quantitative deficiency of antibodies. Although there are a number of laboratory tools that are helpful in the evaluation of patients suspected of having an antibody deficiency disorder, such as quantitative serum immunoglobulin analysis, analysis of lymphocyte subpopulations by flow cytometry, or even genetic diagnoses of single-gene mutations, the evaluation of a patient’s response to immunization with a vaccine and the production of specific antibodies are extremely important. The assessment of qualitative antibody responses is not only important in confirming a specific antibody immune deficiency but is also important in the decision-making process for using immunoglobulin replacement therapy. If on initial evaluation patients demonstrate a deficient quantitative antibody response, an antigen challenge or booster vaccine immunization with existing Food and Drug Administration (FDA)—approved vaccines can determine whether the patient has the ability to generate an adequate qualitative antibody response. In an effort to provide guidance in the evaluation of patients for a humoral PIDD, a working group of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma and Immunology (AAAAI) published a report in September 2012. This article discusses some of the topics related to the use and interpretation of vaccine responses in the evaluation of patients for a possible PIDD.

Strategies and Evidence

Table 1 lists some of the common vaccines that are currently used to measure humoral immune function. Diphtheria and tetanus toxoid vaccines are the most commonly used vaccines to assess antibody production to protein antigens. These vaccines elicit T-cell–dependent antibody responses. Nonconjugated pneumococcal polysaccharide vaccines are commonly used to assess T-cell–independent humoral immune responses. The meningococcal Haemophilus influenzae type b and pneumococcal vaccines are available as conjugates to a protein carrier that changes their immunogenicity to T-cell–dependent responses. These vaccines have different immunologic characteristics that lead to variability in time to peak antibody levels and levels that lead to protection against their specific pathogen.

Use of Pneumococcal Polysaccharide Vaccines in the Evaluation of Humoral Immune Function

Two types of pneumococcal vaccines are available: Prevnar 13 (PCV13), a conjugate vaccine with 13 pneumococcal polysaccharide serotypes, and Pneumovax (23vPPV), an unconjugated purified pneumococcal polysaccharide vaccine of 23 serotypes. PCV13 is part of the routine immunization series of children that starts at 2 months of age. 23vPPV is indicated for individuals 65 years and older and in high-risk pediatric patients, such as those with sickle cell anemia, asplenia, nephrotic syndrome, and other conditions, to reduce the susceptibility to a Streptococcus pneumoniae infection. Healthy individuals immunized more than 5 years previously may have waning antibody levels. Therefore, nonprotected antibody levels in these individuals are generally not evidence of an antibody immune deficiency disorder.

A number of methods are available to measure specific antibodies to pneumococcal polysaccharide serotypes. Currently, the most commonly used techniques for measuring these antibodies use an enzyme-linked immunosorbent assay method or a Luminex assay. The implementation of adsorption methods to remove cross-reactive antibodies to polysaccharides C and serotype 22F have improved the specificity of these assays. The
measurement of specific antibody titers to a minimum of 12 to 14 serotypes, including those serotypes shared between PCV13 and 23vPPV, should be performed 4 to 6 weeks after immunization. However, most commercial laboratories can now analyze all 23 serotypes in the 23vPPV. Baseline or prevaccine antibody measurements allow for the determination of the change or increase in response to the challenge vaccine; prevaccine and postvaccine response should be evaluated in the same commercial laboratory. Functional assays (e.g., opsonophagocytosis) for detecting specific pneumococcal polysaccharide antibodies may provide a better assessment of the quality of the specific antibody response, but these assays are currently only a research tool and are not commercially available.

Assessment of the specific antibody response to the pneumococcal vaccines gives important information: the capacity to produce a protective antibody response and the magnitude of the response or how much specific antibody is made to a particular serotype. These 2 parameters are useful in evaluating a patient’s ability to respond to a vaccine challenge for the evaluation of an underlying immune deficiency. However, the qualitative features of the response to a particular serotype can vary with age and the immunogenicity of the serotype polysaccharide. This complexity in the response to the pneumococcal polysaccharides has not made it any easier to interpret the response of a patient for the evaluation of an immune deficiency. A protective (normal or adequate) response to each pneumococcal polysaccharide serotype is defined as an antibody titer of 1.3 M/C21 g/mL or higher. However, some commercial laboratories have defined their own values as a normal or adequate response, ranging from a low of 1.0 M/C21 g/mL to a high of 2.0 M/C21 g/mL. It is not clear how these commercial laboratories have come to designate a normal or adequate response using these values when the consensus of the clinical immunology community is 1.3 M/C21 g/mL or higher.

Several studies have found that the maximum titers achievable for each serotype may differ from each other and that some pneumococcal polysaccharide antigens are more antigenic than others. For example, polysaccharide serotype 3 is immunogenic even in young children who are unable to respond to serotypes 6B and 23F poor immunogens. As stated, this has led to defining an adequate response based more on experience than rigorous clinical studies. Nevertheless, the number of pneumococcal serotypes after immunization that are protective (e.g., ≥1.3 M/C21 g/mL) can be used to define a normal or adequate response. Although evidence is limited, using published literature the working group has arrived at a consensus that a normal response to an unconjugated pneumococcal polysaccharide vaccine in children from 24 months to 5 years of age is the development of protective titers (≥1.3 M/C21 g/mL) to 50% or more of the serotypes tested and/or at least a 2-fold increase in titer for those serotypes already 1.3 M/C21 g/mL or higher at baseline (before immunization). For individuals 6 to 65 years, a normal response is defined as a conversion of 70% of the serotypes tested and/or at least a 2-fold increase in the titers from baseline.

It is not uncommon for adults and older children to have a pre-vaccination titer (baseline) greater than 1.3 M/C21 g/mL for several pneumococcal polysaccharide serotypes. The higher the pre-immunization titer for a particular serotype, the less likely the titer will have a significant increase after vaccination. In a recent study addressing this issue, Balcas and colleagues found that only 10% to 40% of patients could attain a 4-fold response when the initial titer was greater than 1.3 M/C21 g/mL, depending on the serotype. The probability of a 4-fold increase in antibody titer response decreased as the preimmunization titer increased. In addition, there were serotype-specific absolute preimmunization values above which a 4-fold or greater response would not be expected. This value varied among serotypes and ranged from 4.4 to 10.3 M/C21 g/mL. This held true regardless of age, sex, serum IgG level, or IgG subclass values. Balcas and colleagues simplified this concept by assuming that patients with baseline protective antibody titers can still mount a 4-fold increase in antibody response as long as their preimmunization titer was less than 4 M/C21 g/mL. However, most individuals could increase their vaccine antibody titers approximately 2-fold for most of the 14 pneumococcal polysaccharide serotypes analyzed in the study. Thus, the working group for simplicity suggested for those serotypes in which the prevaccine antibody titer was initially protective (e.g., >1.3 M/C21 g/mL) that a 2-fold response would be considered an good response.

A common question is whether subsequent administration of a vaccine is contraindicated. Development of hyporesponsiveness in adults after repeated polysaccharide immunization has been reported for both pneumococcal polysaccharide vaccines and unconjugated meningococcal polysaccharide vaccines. In our own practice, we have generally waited at least 1 year before repeat administration of a booster immunization with the 23vPPV in children. Several studies have found that the prior administration of a conjugated pneumococcal polysaccharide vaccine can prime the response to the unconjugated purified pneumococcal polysaccharide vaccine. This priming effect is serotype specific in that serotypes not in the conjugate vaccine (PCV13) are not affected. This observation has led in our own practice to children being evaluated for selective antibody deficiency to administer both the 23vPPV and PCV13 at the same time to try to optimize the response, although we do not have any data to confirm this approach. Nevertheless, it does save time in the process of evaluation for the decision of using replacement immunoglobulin therapy. If the vaccine responses to both vaccines (unconjugated and conjugated serotypes) are not sufficient, it makes the decision for a trial of immunoglobulin therapy easier and more robust.

### Table 1

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>T-cell—Independent or —Dependent</th>
<th>Peak antibody levels</th>
<th>Protective level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemophilus influenzae type b conjugate</td>
<td>Dependent</td>
<td>6 mo (3-4 wk after third dose)</td>
<td>1.0 M/C21 g/mL</td>
</tr>
<tr>
<td>Meningococcal conjugate</td>
<td>Dependent</td>
<td>2-4 wk</td>
<td>2 M/C21 g/mL</td>
</tr>
<tr>
<td>Meningococcal polysaccharide</td>
<td>Independent</td>
<td>2-4 wk</td>
<td>2 M/C21 g/mL</td>
</tr>
<tr>
<td>Pneumococcal conjugate</td>
<td>Dependent</td>
<td>4 wk</td>
<td>≥1.3 M/C21 g/mL</td>
</tr>
<tr>
<td>Pneumococcal polysaccharide</td>
<td>Independent</td>
<td>4 wk</td>
<td>≥1.3 M/C21 g/mL</td>
</tr>
<tr>
<td>Rubies</td>
<td>Dependent</td>
<td>21 days after third dose for preexposure prophylaxis</td>
<td>0.5 IU/mL</td>
</tr>
<tr>
<td>Tetanus</td>
<td>Dependent</td>
<td>2-3 wk after initial series</td>
<td>0.15 IU/mL</td>
</tr>
</tbody>
</table>

*Adapted from Orange et al.*

**Use of Novel Vaccine or Neoantigen Vaccines to Measure a Humoral Immune Response in Patients Receiving Replacement Immunoglobulin Therapy**

A number of vaccines have been used as neoantigens to immunize patients for the evaluation of an antibody deficiency while receiving replacement immunoglobulin therapy (Table 2). Some of these vaccines are not available in the United States and/or are investigational only. Nevertheless, this discussion may be useful to the reader to examine these alternatives and their issues.
In the United States there are currently 2 meningococcal vaccines licensed for children 2 years and older and in adults. The more common vaccine used today is the routine conjugate vaccine that is recommended for preadolescent children (MCV4, Menactra [Sanofi Pasteur Inc, Swiftwater, Pennsylvania], and Menevo [Novartis Vaccines and Diagnostics Inc, Emeryville, California]). Both vaccines contain 4 serogroups (A, C, Y, and W-135) to Neisseria meningitides. A 4-fold or greater increase in specific antibody to at least 2 of the serogroups is believed to be a adequate response in healthy individuals; antibody levels peak at approximately 4 weeks after immunization. However, because the meningococcal vaccine is now a routine vaccine for adolescent children, we can expect that going forward pools of immunoglobulin product will contain substantial amounts of specific antibodies to the N meningitides serogroups. This will make the meningococcal vaccines not as useful as a neoantigen or novel vaccine in the assessment of a specific antibody response in patients already receiving immunoglobulin replacement therapy. Another potential alternative neoantigen or novel vaccine is the rabies virus vaccine. There are 2 cell culture rabies virus vaccines available in the United States: a human diploid cell vaccine (Imo-vax; Aventis Pasteur, Lyon, France) and a purified chick embryo cell vaccine (RabAvert; Novartis Vaccines and Diagnostics). The regimen for preexposure prophylaxis is administration of the vaccine at 0, 7, and 21 or 28 days. The rabies vaccine prepared from purified chick embryo cell culture appears to be better tolerated with fewer adverse effects than the rabies virus vaccine derived from a human diploid cell culture. An adequate protective antibody response is a titer of 0.5 U/mL or complete virus neutralization at a 1:5 serum dilution. Healthy individuals given these rabies vaccines make an IgG antibody response but also specific IgM and IgA antibodies. The vaccines also elicit a T-cell immune response that can be evaluated by lymphocyte proliferative responses to rabies virus antigen. However, this T-cell lymphocyte proliferative response, in part, may be related to a superantigen effect of the rabies virus nucleocapsid protein antigen. The number of immunizations needed to produce a satisfactory humoral immune response can be a major issue for these vaccines in the evaluation of the PIDD patients. For example, a primary immunization with one booster 3 months later is enough to lead to a satisfactory protective level of IgG antibodies in healthy individuals. However, this timetable may be unacceptable in the evaluation of a PIDD patient. Currently, not enough information is available to determine whether one single rabies virus vaccine dose can discriminate between healthy individuals and those with PIDD. There are several reports on the use of the rabies vaccine to evaluate patients for a humoral immune deficiency. For example, a 10-year-old Turkish girl with a mutation in the CD19 gene who was receiving intravenous immunoglobulin replacement therapy was given a primary immunization and a single secondary booster 13 weeks after the initial rabies virus vaccine. Although the patient developed a low normal specific IgG antibody response to the rabies virus vaccine after the primary immunization, the response after the secondary immunization at week 13 was abnormal. The potential for adverse reactions and the paucity of data in PIDD patients make the rabies vaccine less than desirable to use as a neoantigen for the evaluation of patients for a humoral immune deficiency.

Another novel or neoantigen vaccine that could be useful in PIDD patients receiving immunoglobulin replacement therapy is the tickborne encephalitis virus vaccine. Seidel and colleagues used this vaccine in Europe to evaluate the specific IgG antibody response in PIDD patients receiving immunoglobulin therapy. US plasma-derived intravenous immunoglobulin does not have neutralizing antibody to tickborne encephalitis virus. The tolerability and safety of this vaccine make it a good candidate vaccine for the evaluation of humoral immune function in a patient receiving immunoglobulin replacement therapy. However, this vaccine is not available in the United States.

Another truly neoantigen vaccine is the bacteriophage ϕχ174. This neoantigen has been used for the evaluation of the primary and secondary humoral immune responses. This vaccine can also be used to assess isotype class switching and the kinetics of the antibody response. Although this vaccine is useful in the evaluation of antibody production in patients with suspected humoral immune deficiency receiving immunoglobulin replacement therapy, ϕχ174 vaccine is not an FDA-licensed vaccine and at present can only be used on a research basis.

### Areas of Uncertainty

Many variables may influence the humoral immune response including the age of the patient, the type of vaccine used, the tests used to measure the antibody response, and the interpretation of the laboratory results. Although a value of 1.3 μg/mL or greater of specific antibody to a pneumococcal polysaccharide serotype is thought to be an antibody level that is protective against invasive (sepsis) S pneumoniae infection, it is not known what an adequate or protective antibody level is for an infection of a mucosal space, such as in the sinuses.

We all have observed patients who respond well to 23vPPV and have a decrease in the number and frequency of infections but subsequently start to have recurrent infections, mainly sinusitis, again. Additional laboratory evaluation for specific pneumococcal antibodies reveal markedly decreased levels of specific antibody within 6 months of the prior immunization. This clinical phenotype has been referred to as selective antibody deficiency of the memory phenotype. These patients usually respond to an additional administration of 23vPPV. Leiva et al reported that many of these patients have low IgM- and/or class-switched memory B-cells. The long-term consequences of these low memory B-cells and their prognostic implications remain to be determined.

### Guidelines

This section summarizes some of the important points of using vaccines to evaluate patients for a specific antibody deficiency. A more expanded list and discussion of summary statements and guidelines can be found in the AAAAI working group report by Orange et al.

- **Live viral vaccines should be avoided in patients with certain immune deficiencies.** For example, patients with deficiencies or impairment in cell-mediated immunity (eg., severe combined immunodeficiency disease) or patients with antibody deficiency (eg, X-linked agammaglobulinemia, common variable virus, and IgA deficiency) who have an increased susceptibility to enteroviruses should not be given live viral vaccines.
- **Some PIDDs, such as severe combined immunodeficiency virus or X-linked agammaglobulinemia, that are usually diagnosed early**

### Table 2

<table>
<thead>
<tr>
<th>Vaccine to assess B-cell function in patients receiving IgG replacement therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningococcal polysaccharide</td>
</tr>
<tr>
<td>Conjugated</td>
</tr>
<tr>
<td>Unconjugated</td>
</tr>
<tr>
<td>Rabies virus vaccines</td>
</tr>
<tr>
<td>Can be used as a neoantigen in patients receiving IVIG</td>
</tr>
<tr>
<td>Tickborne encephalitis virus vaccine</td>
</tr>
<tr>
<td>Can be used as a neoantigen in patients receiving IVIG1</td>
</tr>
<tr>
<td>No tickborne encephalitis vaccines are licensed or available in the United States</td>
</tr>
<tr>
<td>Bacteriophage ϕχ174</td>
</tr>
</tbody>
</table>

Abbreviation: IVIG, intravenous immunoglobulin.

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in life because of extensive immune abnormalities in T-cell and/or B-cell immunity need not undergo vaccine antigen challenge to document impairment in specific antibody production because it would delay necessary therapy and would provide little additional value.

- Nonconjugate polysaccharide vaccines should not be a component for the routine investigation of antibody deficiency in children younger than 18 months while they are receiving their primary immunization series.
- Immediate repeat booster immunization with unconjugated polysaccharide vaccines is not recommended and might promote hyporesponsiveness.
- A protective (normal or adequate) response to each pneumococcal polysaccharide serotype is defined as an antibody titer of 1.3 μg/mL or higher.
- A normal response to an unconjugated pneumococcal polysaccharide vaccine in children from 24 months to 5 years of age is the development of protective titers (≥1.3 μg/mL) to 50% or more of the serotypes tested and/or at least a 2-fold increase in titer for those serotypes already 1.3 μg/mL or higher at baseline (before immunization). For patients 6 to 65 years, a normal response is defined as a conversion of 70% of the serotypes tested and/or at least a 2-fold increase in the titers from baseline.
- For those serotypes in which the prevaccine antibody titer was initially protective (eg. >1.3 μg/mL), a 2-fold response would be considered an adequate response.
- A diagnosis of specific antibody deficiency is defined as a deficient response to 23vPPV. The responses to protein or conjugate vaccines are intact. Serum immunoglobulin levels are normal, although some patients may have decreased serum IgG subclass levels. Immunoglobulin replacement therapy in these patients can be considered, depending on the clinical presentation (eg, history and nature of infections), the response to prophylactic antibiotics, and optimal management of other comorbid conditions (eg, allergy).

Conclusions and Recommendations

The use of vaccines in the immune evaluation of patients for PIDD is a valuable tool. Vaccine immunizations are not only useful in characterizing the type and extent of the humoral immune abnormality but are a critical component in seeking approval from payers for the use of replacement immunoglobulin therapy. The 23vPPV is still our most valuable nonprotein, polysaccharide vaccine for the evaluation of a T-cell—indepedent response. However, as new conjugate vaccines for pneumococcal polysaccharides are developed with coverage of more serotypes, the use of the 23vPPV will be less useful as a diagnostic tool. In addition, we are still rigorously discussing what constitutes a normal response in different age groups to the pneumococcal polysaccharides antigens. Although there are several novel neoantigen vaccines available, more data are needed on the use of these vaccines in PIDD patients, especially those undergoing immunoglobulin replacement therapy, and the laboratory methods for the quantification of the antibody response outside the research laboratory. Nevertheless, it is important to remember that vaccine responses are only one part of the evaluation of a patient with PIDD. The history, physical examination, other laboratory parameters, and finally advances in genetic testing are critical in helping immunologists in the diagnosis and treatment of patients with PIDD. Although the report provides evidence-based information, opportunities still remain to generate new information on use of vaccines as diagnostic tools for the diagnosis of patients with antibody or humoral immune deficiency.

References