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Graphical Method for Evaluating Antibody Response to Vaccines

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We describe a technique which facilitates the presentation, analysis, and interpretation of antibody responses elicited by vaccines or other immune stimuli.

Three methods are commonly used when analyzing the antibody response to vaccines or other immune stimuli. The first is the ratio of the post-immunization to the preimmunization antibody level, or "fold-rise" (1, 2, 8). Its disadvantage is that the actual concentration of antibody achieved, which may relate to protective efficacy (3, 5, 9), is not specified. The second method is the calculation of the mean antibody concentrations for the population before and after immunization (2–4, 6–8). Usually geometric means are used because the logarithms of the antibody concentrations are more nearly normally distributed than the arithmetic values. However, the variation in antibody concentrations and in antibody responses is difficult to convey with this method. The third and simplest method tabulates the proportion of patients who reach an antibody level thought to be protective (3, 4, 9); the actual concentrations and fold-rises are not presented.

We propose a graphical method for the presentation and evaluation of antibody responses which conveys the full information inherent in such data.

Figure 1 illustrates the plotting method. The horizontal and vertical axes are the pre- and post-immunization antibody concentrations, respectively, plotted on a logarithmic scale. Each patient's pre- and post-immunization antibody level generates a single point on this graph. A line drawn through points of equal concentration on both axes corresponds to "no response." The line drawn parallel to and above the no-response line in Fig. 1 corresponds to a twofold rise in antibody concentration. Additional parallel lines may be added to indicate fourfold, 10-fold, etc. rises or falls in antibody. The horizontal reference line indicates the protective antibody level, when this has been established or arbitrarily defined.

Several hypothetical responses, A to F, are illustrated in Fig. 1. Dotted arrows projected vertically to the no-response line indicate the changes in antibody concentration elicited by the vaccine. Response A, indicating an individual with a low antibody level before and the same level after immunization, falls on the no-response line. Responses B and C both represent fourfold rises in antibody level and thus fall above the twofold response line. However, response B did not achieve the hypothetical protective level because of the low preimmunization antibody concentration. Response D shows a less than twofold rise in antibody which might be considered an inadequate response to the vaccine. However, since both pre- and post-immunization levels are well above the protective level, this should not be interpreted as a vaccine failure. Responses E and F show decreases in antibody concentrations which are occasionally observed, especially in immunologically impaired populations (6). Again, the significance of the fourfold decreases differs since individual F's antibody concentration remains above the protective level, and individual E's falls below the protective level. The method thus portrays each patient's response (fold-rise) in the context of the absolute antibody concentration which is vital in evaluating the significance of the response.

Figure 2 illustrates the response of 28 adult volunteers to two of the serotypes in a Lilly dodecavalent pneumococcal vaccine. The response to serotype 6 (panel A) is representative of the responses to most of the other serotypes in the vaccine. The fold-rise in antibody is similar over a wide range of preimmunization antibody concentrations. Consequently, the patients with low preimmunization antibody levels who were thought to be at highest risk of infection tended to have the lowest post-immunization levels. In particular, the four individuals who failed to obtain antibody levels of 200 ng/ml (panel A, dotted circle) had the lowest preimmunization antibody level. Although this is dis-

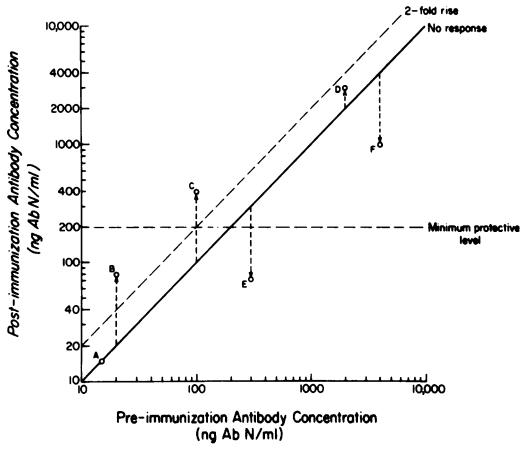

FIG. 1. *Method for plotting antibody concentrations before and after immunization in six hypothetical patients, A to F.*

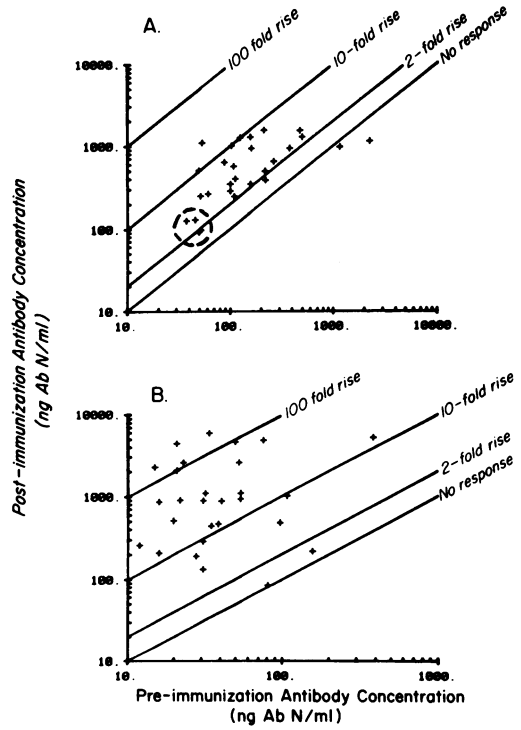

FIG. 2. *Antibody concentrations of 28 adult volunteers before and after immunization with dodecavalent pneumococcal vaccine. Panel A displays the response to pneumococcus type 6; panel B displays the response to pneumococcus type 8.*

quieting, one cannot conclude that these responses are inadequate unless the level of antibody necessary for protection can be defined. Another feature typical of many serotypes is that individuals with high preimmunization antibody levels have little change or even a decrease after immunization (Fig. 1, panel A). Unless the magnitude of the fall is great, however, such responses are unlikely to have clinical significance with respect to protection and should not be considered vaccine failures.

Figure 2, panel B, shows the antibody response of the same group of volunteers to serotype 8. The majority of individuals achieved antibody concentrations greater than 200 ng/ml and increases greater than 10-fold. In contrast to serotype 6, the lowest post-immunization antibody levels were not obtained by the individuals with the lowest preimmunization levels. Panel B demonstrates clearly that the greater immunogenicity of serotype 8, as measured by fold-rise in antibody, is due partly to the low preimmunization antibody levels and partly to a substantial number of post-immunization levels greater than 1,000 ng/ml.

Figure 3 illustrates the antibody levels to serotype 4 of a more complex population before and after immunization with Lilly dodecavalent pneumococcal vaccine. The majority of the individuals were splenectomized and had completed treatment for Hodgkin's disease as previously described in detail (6). With symbols for the various treatments, the graph clearly shows that the most intensively treated groups (total nodal radiation ± chemotherapy and subtotal radiation + chemotherapy) had the lowest antibody levels before immunization and the lowest responses to the vaccine. Particularly disturbing is that several of these patients had decreases in antibody concentration after immunization.

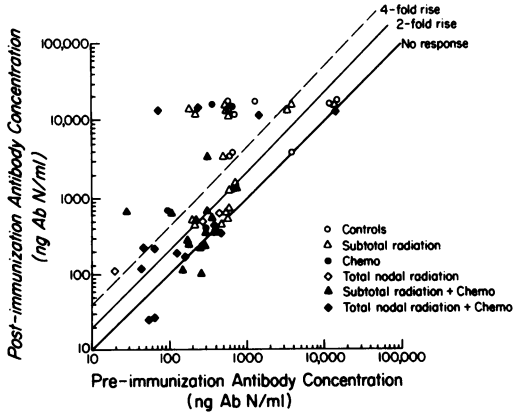

FIG. 3. *Antibody concentrations to pneumococcus type 4 of 53 patients with Hodgkin's disease and 10 controls before and after immunization with dodecavalent pneumococcal vaccine. The symbols indicate the treatment for Hodgkin's disease which had been completed before immunization.*

Figure 4 demonstrates that this method can be adapted to examining the persistence of antibody after immunization (3). Antibody levels of the Hodgkin's disease patients 3 weeks and 6 months after immunization were used to generate the plot. Points falling below the no-change line indicate decreases in antibody concentrations which occurred during this period. The majority of individuals had less than twofold decreases in antibody during the 6 months after immunization. Greater than threefold decreases occurred only in patients with Hodgkin's disease. Also evident is a single patient who had a delayed increase in antibody level to type 3 as well as to other serotypes (6).

Figure 5 shows the distribution of antibody levels to type 3 pneumococcal polysaccharide in 43 adult volunteers before and after immunization with Lederle bivalent Influenza A/Port Chalmers B/Hong Kong split-product vaccine.

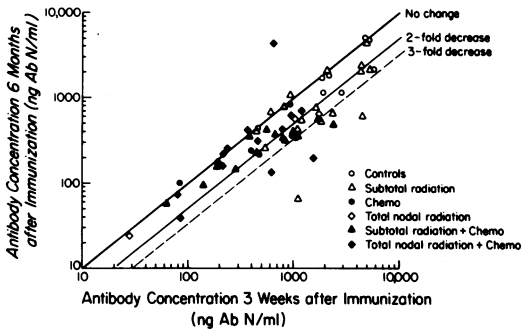

FIG. 4. *Antibody concentrations to pneumococcus type 3 of 53 patients with Hodgkin's disease and 10 controls 3 weeks and 6 months after immunization with dodecavalent pneumococcal vaccine.*

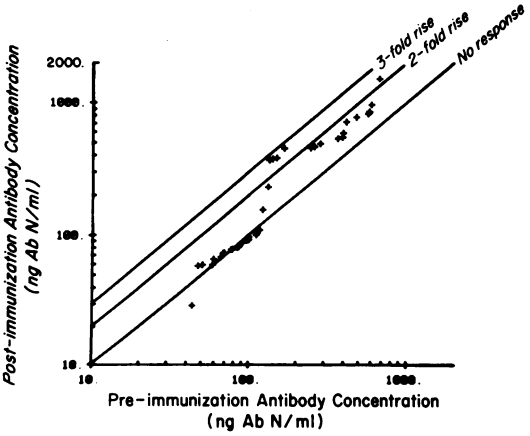

FIG. 5. *Antibody concentrations of 43 adult volunteers to pneumococcus type 3 before and after immunization with bivalent Influenza A/Port Chalmers B/Hong Kong split-product vaccine.*

Clearly evident are small but consistent increases in antibody concentrations which are confined to individuals with preimmunization antibody levels greater than 120 ng/ml. One possible explanation for this observation is that the antigens of influenza virus and pneumococcus type 3 cross-react. Another is that the individuals with higher preimmunization antibody levels received a nonspecific stimulus for antibody production from the influenza vaccine. The point relevant to this discussion is that this relationship is readily recognized and illustrated by the plotting method.

The graphical method proposed here for evaluating the antibody response to vaccines possesses several advantages over methods of analysis described earlier. Both the fold-change and the absolute levels of antibody are presented in an easily visualized manner. Large numbers of observations can be presented on a single plot, and the use of symbols permits direct comparisons between different populations. The antibody concentrations of each individual can be extracted from the plot and may thus be reinterpreted in the light of new information, such as guidelines for minimum protective levels. Variation in response and unusual responses such as decreases in antibody levels are clearly apparent. The responses to a given antigen with different vaccine preparations may be plotted on the same scale and readily compared by overlay on a light table. Large numbers of plots, as with the multivalent pneumococcal vaccines, are easily generated with user-oriented data analysis systems such as the PROPHET system used in this study. Finally, the method may also be applied to antibody titers (which we call titer plots) instead of concentrations, or generalized to other biological data which change in response to some stimulus or with respect to time.

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