Appendixes

Appendix A.— Relationship of Serum Antibody Concentration to Incidence of Influenza Illnesses*

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The most instructive period for studying the relationship between immunity to influenza and levels of antibody produced by inactivated vaccines occurs on those rare occasions when vaccine produced from a major new antigenic subtype of influenza A is administered to individuals before their first infection with this new pandemic subtype, This opportunity has occurred only three times in over **40** years, i.e. in 1957 (H2N2 virus), in **1968 (H3N2** virus), and in 1978 (when H1N1 strains of influenza caused illnesses primarily among individuals born after 1957). Much useful information also may be obtained during interpandemic periods, but primed individuals respond better to vaccine than do unprimed subjects. The study of artificially induced infections of human volunteers may also yield useful information, but the virus challenge may not resemble that which occurs naturally, and thus interpretation of results is difficult.

The relationship between the concentration of influenza serum antibody and immunity to influenza infection or illness may be influenced by a number of variables. Serum antibody formed after a first infection with an antigenically new subtype of influenza A may be quantitatively and qualitatively different from antibody produced after several sequential infections with the same subtype of influenza A. The initial infection may produce a relatively low concentration of serum antibodies, but subsequent infections may boost antibody concentrations which may persist at measurable levels for a longer period of time. Repeated infections with familiar strains of influenza virus may stimulate some antibodies which are specific for the current infecting strain, and other antibodies which are more cross-reactive with earlier strains infecting the same individual. The method of measuring antibody in serum is also an important variable, since hemagglutination-inhibition (HI) and neutralization tests, and other tests such as single radial hemolysis of ELISA, may measure antibodies having somewhat different specific reactivities with influenza viral antigens. While both HI and neutralization tests primarily measure the biological function of hemagglutinin attachment to cell receptors, the single-radial hemolysis tests and enzyme immunoassay (EIA) measure antibodies against both hemagglutinin and neuraminidase, and *in* the **case** of EIA, common internal antigens as well. Although the HI test is the most universally accepted for measurement of serum antibodies in influenza, the actual dilution of serum which will cause complete inhibition of red blood cell (RBC) hemagglutination may vary somewhat depending on the avidity of the hemagglutinin-antibody interaction, as well as other variables such as the method used for removal of serum inhibitors and the type of RBC used.

When examining the relationship between serum antibody and immunity to influenza, it is important to specify whether antibody has been produced by parenteral administration of inactivated vaccine or by natural infection. While serum antibody induced by parenteral vaccination may reflect systemic immunity to virus infections of the lower respiratory tract (lung), resistance to the initiation of influenza virus infection at the mucosal surface of the upper respiratory tract may correlate better with the presence in secretations of virus-specific IgA, induced by replication of the virus at these sites during natural infection.

In 1957, one dose of 200 or 500 chick cell agglutination (CCA) units of inactivated A/Japan/305/57 vaccine was given to volunteers, and **2** to 4 weeks later an A/Japan/305/57-like live virus challenge (diluted nasal washings of infected boys) was given to both vaccinated and unvaccinated volunteers (1). of 33 unvaccinated individuals, 23 (78 percent) developed febrile influenza-like illness while 14 (44 percent) of those given vaccine developed similar illness, Attack rates varied inversely with HI antibody titers induced by vaccination, with rates of febrile illness of 60, 43, and 25 percent, respectively, in 10 individuals with titers <10, 14 individuals with titers of 10 or 20, and 8 individuals with titers ≥ 40. Conflicting data were obtained by Rose and Fukumi in 1957. Rose demonstrated that 200 or 750 CCA units

● NOTE: Reference citations for app. A refer to the **list** of references at the ***end*** of app A.

of monovalent A/Japan/305/57 vaccine provided some immunity to infection during an Asian influenza outbreak (60 to 78-percent efficacy), yet he was unable to demonstrate a serum HI response to the vaccine; a CF antibody rise did occur (2). Fukumi, on the other hand, reported that vaccines induced good HI antibody responses, and infections occurred rarely among individuals with HI titers of 32 or greater (although it is not entirely clear if he refers to antibody induced by vaccination or prior infection)(3).

In 1968, Dowdle, et al., demonstrated that 3,000 CCA, but not 300 CCA, units of inactivated influenza A/Hong Kong/68 vaccine conferred a protective effect against natural challenge with homologous virus (4). Although febrile influenza-like illness occurred among individuals with HI titers of **80** to **160,** illness severe enough to cause patients to remain in bed occurred in only approximately 11 percent of those with HI titers of 80 to 160, compared with an incidence of illness requiring bed rest of about 32 percent in those with titers ≤ 10. (Incidentally, when both HI and neuraminidase-inhibiting (NI) antibody were present at any titer, illness requiring bed rest was seen even less frequently.)

During an epidemic of A/Brazil /11/ 78(HlNl)-like virus infections among university students in Georgia during early 1979, Noble, et al., found that A/USSR/77 vaccine produced a protective effect. Two doses of inactivated vaccine containing 7 mcg of hemagglutinin were given to October and November 1978, and an epidemic occurred in January and February 1979. Among vaccinated individuals with HI antibody titers of ≤ 20 to the epidemic strain, the incidence of influenza-like illness (fever or feverishness and chills with respiratory symptoms and a significant rise of HI antibody to H1N1 virus) was **20** percent (19 of 97), whereas among individuals with titers ≥ 40 only 6 percent (4 of 72) had similar illnesses. The incidence of illness among placebo recipients, all of whom had HI titers ≤ 20, was 25 percent (45 of 179).

From the preceding review it is apparent that great variation is seen in different situations between the level of serum antibody and evidence of immunity. However, approximately a **60-** to 80-percent reduction in typical influenza-like illnesses is generally seen when serum HI antibody titers of 40 to 80 are achieved by vaccination during the first wave of a new major antigenic variant, when attack rates of a placebo group or individuals with titers of 20 or less after vaccination are used for comparison. Additional data have been generated by investigators who have examined serum antibody tiers and the resistance to an artificial challenge with intranasally administered live influenza virus. Hobson, et al., reviewed experience at the Common Cold Unit, Salisbury, England **(570** volunteers challenged with influenza "A2 viruses" and **462** volunteers challenged with influenza B viruses) and found that serological evidence of infection occurred rarely among individuals with prechallenge HI titers of 100 to 200 (5). At titers of 48, serological evidence of infection was reduced approximately 60 to 80 percent when compared with infection rates in those with prechallenge homologous HI serum antibody titers of 6 to 12.

During interpandemic periods, a similar correlation between serum HI antibody titers and protection has also been observed. Among vaccinated and unvaccinated individuals, the incidence of influenza-like illness was approximately 15 to 20 percent among those with titers ≤ 16, whereas attack rates were 3.5 percent or lower in those with titers of ≥ 32 (6). Likewise, Meiklejohn, et al., demonstrated a good correlation between serum HI antibody titer and the probability of clinical infection with A/FM/1/47-like viruses (7). The estimated incidence of infection among those with titers <8 was 18.3 percent, compared to a 1.6-percent infection rate among individuals with titers of 32; no influenza-like illnesses were reported among those with titers ≥ 64. Others have reported generally similar findings (8,9,10), although the method of expressing serum dilutions in early papers may result in titers higher than titers obtained with methods now in use, and thus a comparison of actual titers may be misleading (6).

It is clear from the review of existing data that no single titer of serum influenza HI antibody can be chosen to indicate any specific index of immunity to natural challenge. This is particularly true during the first appearance of a new major antigenic variant of influenza A, because the data available are limited, It is clear, however, that an increasing concentration of homologous serum HI antibody confers an increasing degree of protection against typical influenza illness. This appears to be true whether the antibody is induced by vaccination or by natural infection. Thus, HI antibody titers of **40** to 80 (or 32 to 64) have generally been found to provide a reduction in the incidence of typical influenza-like illness of 60 to 80 percent, when compared with the incidence of illness n those with tiers ≤ 20 or ≤ 16.

The question of what concentration of serum antibody is to be considered ideal following vaccination is, therefore, a matter of judgment, as stated by Salk 20 years ago (11):

> The incentive for achieving the highest levels that are practicably attainable is clear. In answer to the question as to how high the level should be, it might be said that the higher the better; the higher the level

attained, the greater the persistence at the more effective levels.

The limiting factors in attaining the highest level are clinical and economic. If one eliminates the economic considerations, the upper limit attainable, using a single dose of vaccine, is set by the frequency or severity of systemic reactions that accompany the use of concentrated virus preparations; in infants and young children the "severity" of the reaction is perhaps of greater consequence, and in the adult population the "frequency" of reactions is of first importance.

Appendix A References

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