Inactivated influenza virus vaccines (IVVs) were approved for use in humans ~50 years ago. Since that time, they have been used with success for prevention of influenza by the military and among civilians at high risk for development of severe disease and life-threatening complications from infection [1]. The latter group include elderly persons and those with a number of chronic conditions. Although a recommendation for annual use of vaccine in these populations was in place for decades, vaccination rates were relatively low until about 1990 [2]. In recent years, doses prepared by manufacturers for the US population and rates of immunization among high-risk persons have progressively increased (Williams M, personal communication). This is occurring without a change in the properties of available vaccines; they have been basically the same (except for periodic change in antigens) for more than a decade. At present, these vaccines are the most commonly used vaccines in the United States and most other developed countries. Moreover, they are currently the major potential preventative for pandemic influenza. It is important that efforts to improve IVVs continue so that both the record and the reputation of their value are unquestioned.

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Vaccine Evolution

IVVs have evolved over the decades since their introduction into public health. Major features of this evolution are summarized in table 1. It was shown early that chick embryo–grown virus was a satisfactory source of virus for vaccine and that formaldehyde treatment preserved antigenicity while destroying infectivity. Demonstration that serum antibody responses to vaccine were followed by protection against an infectious challenge was provided shortly thereafter. After it was shown that virus hemagglutination and that antibody responses can be measured in hemagglutination-inhibition (HAI) tests, quantitative hemagglutination assays became the potency standard for vaccine preparations. Reactogenicity of vaccine preparations, particularly early vaccines, was directly related to dose. Split-product vaccines were developed primarily to reduce this reactogenicity [3], and various other means for removal of nonviral contaminants of vaccine preparations were introduced to reduce reactogenicity.

Reports on the numerous factors that can influence immunogenicity probably make IVVs the most studied vaccines over the past 50 years (table 1). Properties of the vaccines as well as characteristics of the host have been thoroughly assessed. Of note in these numerous reports are the repeated demonstrations of increasing serum HAI antibody responses with increasing vaccine dose, the requirement for at least two doses for satisfactory responses among unprimed persons, and that both humoral and cellular systemic immune responses and antibody responses in respiratory secretions follow parenteral administration in primed vaccinees. Immunizations by the respiratory and intestinal routes have been tested in an attempt to improve antibody responses in secretions.

Protection against infection has repeatedly, but not always, followed vaccination. When demonstrable, the degree has invariably related directly to titers of specific serum anti-HA antibody. If the infectious challenge is with a virus that is related but antigenically distinct from the vaccine virus, then the titers of serum anti-HA antibody and the degree of protection are lower than if both challenge and vaccine virus are antigenically similar. The degree of protection against natural
Table 1. Major changes and observations that characterize the development and study of inactivated influenza virus vaccines since 1993.

<table>
<thead>
<tr>
<th>Preparation and standardization</th>
<th>Reactogenicity</th>
<th>Immuneogenicity</th>
<th>Protection</th>
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<td>Preparation and standardization</td>
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<tr>
<td>Formaldehyde inactivation</td>
<td>Relation to dose</td>
<td>Repeated demonstrations</td>
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<tr>
<td>Chick embryo source</td>
<td></td>
<td>Relation to serum antibody</td>
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<tr>
<td>Hemagglutinin standard</td>
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<td>Antigenic variation</td>
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<td>High-growth reassortants</td>
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<td>Adjuvant enhancement</td>
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<td></td>
<td></td>
<td>Age and various diseases</td>
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<tr>
<td></td>
<td></td>
<td>Antibody in secretions</td>
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<tr>
<td></td>
<td></td>
<td>Neuraminidase</td>
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<tr>
<td></td>
<td></td>
<td>Cell-mediated immune response</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Mucosal immunizations</td>
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</tbody>
</table>

infection varies considerably for the different epidemics, and this variation is partly explained by these antigenic differences between vaccine and epidemic virus.

There are a number of recurring findings over the decades of study that deserve emphasis when considering improvement of inactivated vaccines. Among these are that purification reduces reactogenicity; immunogenicity can be improved; and protection against infection and illness, which is frequently low, regularly follows vaccination. Clearly, an opportunity for improving IVVs exists. While this is desirable at present, it could be necessary for satisfactory effectiveness in the face of pandemic influenza.

Current Vaccines

The major deficiency of current vaccines is that the protection they induce is less than desirable; it may be high among healthy adult populations but is generally lower among young children and the elderly. Moreover, the effectiveness is of short duration, which, together with antigenic changes in the virus, form the basis for the recommendation for annual immunization. A potential requirement for two doses of vaccine for large numbers of persons in the event of a potential new and unique pandemic virus is another disadvantage of these vaccines.

There are still other disadvantages for which improvement is desirable, such as the need for parenteral administration and limitations in substrate availability, but they are less important than those that contribute to vaccine efficacy.

The variation in efficacies of current vaccines tested in randomized, placebo-controlled field studies in Houston from 1983 to 1990 [4–7] (Couch RB, et al. and Keitel WA, et al., unpublished data) is shown in table 2 along with the results of Govaert et al. [8] among persons ≥60 years of age, the only placebo-controlled study published for this age group. All persons in those studies were considered healthy, including those in the study by Govaert et al., although many in that study were later determined to have underlying disease. Infection was detected by virus isolation or serum antibody increase (or both), with most infections in each study detected by serology. Because of the reduction in frequency of antibody responses among infected persons with increasing titers of preexposure antibody, a feature of vaccinated populations, actual protection rates are probably somewhat lower than those shown [9]. The reduction in rates of infection-associated respiratory illnesses parallels the rates of reduction of infection but may be somewhat greater or lesser in a given study.

As shown in table 2, protection against infection varied between 0% and 100% among the various studies and age groups. Lowest protection levels were seen among children 3–5 years of age, and the highest levels were seen among healthy adolescents and young adults. In addition, lower protection rates were seen more often when the epidemic virus was heterotypic to the vaccine virus. Thus, although effective for prevention of infection with an influenza virus, vaccine-induced protection may be low.

Mediators of Immunity

A consideration of what is needed to improve effectiveness of IVVs must focus on our understanding of mediators of immunity to influenza virus infection. Vaccine-induced prevention of infection requires induction of antibody to the viral hemagglutinating protein (HA) in both serum and respiratory secretions [10, 11]. A reduction in intensity of infection so that illness does not occur or is ameliorated is the primary function of antibody to the neuraminidase (NA) antigen, although anti-HA antibody can exhibit a similar function [12, 13]. An early T cell cytotoxic (CTL) response could also contribute toward reducing the intensity of the infection, but at present, its major

Table 2. Efficacy of inactivated influenza virus vaccine—Houston randomized control studies, 1983–1990.

<table>
<thead>
<tr>
<th>Age group (years)*</th>
<th>No. of epidemics</th>
<th>% decrease in infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–5</td>
<td>2</td>
<td>0, 27</td>
</tr>
<tr>
<td>6–10</td>
<td>2</td>
<td>32, 91</td>
</tr>
<tr>
<td>11–18</td>
<td>2</td>
<td>100, 100</td>
</tr>
<tr>
<td>18–35</td>
<td>3</td>
<td>41, 85, 95</td>
</tr>
<tr>
<td>30–60</td>
<td>5</td>
<td>24, 49, 55, 65, 72</td>
</tr>
<tr>
<td>≥60</td>
<td>1</td>
<td>50</td>
</tr>
</tbody>
</table>

* Data from [4–7].
^ Two doses at 1-month interval.
† From Govaert et al. [8].
role is considered to be mediation of recovery from the infection [10, 11]. Other specific immune mechanisms, such as antibody-dependent cell cytotoxicity and nonspecific immune mediators (e.g., interferon), undoubtedly contribute to control of the infection.

Parenteral administration of IVV leads to induction of anti-HA and anti-NA antibody in both serum and respiratory secretions. An increase in T cell cytotoxicity that can be stimulated in vitro can also follow administration of IVV among adults and children [14, 15]. Although higher CTL responses have corresponded with reduced viral shedding, a persisting and beneficial effect of this response has not been demonstrated in humans vaccinated with IVV [16]. Thus, at present, the focus for an early improvement in IVV is on increasing the anti-HA and anti-NA antibody responses.

**Improving IVVs**

Major options for improving IVVs in the near future include purification of subunits and production of pure proteins via recombinant DNA technology so as to permit administration of increased doses of HA and NA without encountering significant reactogenicity; the use of adjuvants to increase immune responses; the use of immunomodulators to enhance or restore optimal immune responses, particularly for elderly and immunocompromised persons; and the addition of mucosal administration so as to improve the induction of secretory IgA antibody and the amount of antibody in respiratory secretions.

**Dose.** We have recently reevaluated increasing the dose of HA, using purified HA vaccine preparations provided by Pasteur-Mérieux–Connaught Laboratories (Swiftwater, PA). Figure 1 shows serum neutralizing antibody responses to graded doses of HA in an A/Taiwan/86 (H1N1) vaccine given once intramuscularly to healthy college students [17]. As noted, a direct correlation was detected between dose and mean titer that persisted for 24 weeks among persons selected for little or no prevaccination serum antibody as well as those selected for presence of high titers. Of interest is that a maximal response was not identified. The proportion of students with a rise in specific antibody in nasal wash (NW) specimens at 2 weeks is shown in figure 2. The proportion of both IgA and IgG antibody responses increased with increasing vaccine dose. Minimal reactogenicity was noted for these preparations.

The same vaccine preparation was given at the 15-, 45-, and 135-μg dose levels to healthy persons >65 years old (mean, 72) along with similar doses of split-product vaccine [18]. With increasing doses, each vaccine induced an increase in serum HAI or neutralizing antibody and IgG or IgA antibody in nasal washes. Between the 15- and 135-μg doses, both serum and nasal wash response frequencies doubled, and mean titers increased 2- to 3-fold. The split-product vaccines were somewhat more reactogenic, a finding that could restrict formulation of trivalent preparations at the 135-μg dose level for each of 3 virus strains. In a study by Powers et al. [19], serum antibody responses among healthy young adults given recombinant DNA–produced HA vaccines were followed by evidence of protection against natural infection in the vaccinated group.

We have also evaluated purified NA vaccines in a similar manner among healthy young adults [20]. Doses of 2.6–69.6 μg of NA increased frequencies of serum NA-inhibiting antibody responses from 40% to 90% and ELISA antibody from 15% to 80%. Frequencies for a commercial vaccine containing an estimated 7 μg of NA protein were 60% for NA-inhibiting antibody and 55% for ELISA responses.

Thus, it appears that highly pure vaccine preparations of HA and NA can be safely given in increased doses and that these increased doses induce increased frequencies and levels of anti-HA and anti-NA antibodies. Moreover, this increase in response in relation to dose persisted for months after vaccination. Of interest is that an increase in antigen dose does not lead to a proportionate increase in the antibody response, an observation made in earlier studies [21, 22]. Whether these increases in antibody lead to increased protection is yet to be shown; however, similar dose-related increases have done so in the past [21].

**Adjuvants and immunomodulators.** Adjuvants substantially enhanced serum HAI antibody responses of vaccines used during the 1940s and 1950s; both alum and water in oil emulsions were used [23–27]. Large numbers of military recruits were given IVV in mineral oil adjuvant, and significant increases in serum antibody titers occurred. However, although infrequent, occurrences of sterile abscesses at the site of injection led to discontinuation of the use of these vaccines. An alternative, highly immunogenic adjuvant emulsion (adjuvant 65) was never adopted for general use [26]. Recent studies with IVV, using newer adjuvants, have not shown increased magnitudes of antibody responses similar to those reported earlier. Incorporation of an A/H1N1 antigen in liposomes induced little to no increase in antibody responses compared with responses from standard vaccine among either young adults (Cate TR, unpublished data) or elderly persons [28]. A new oil-and-water emulsion using squalene and detergents (MF59) enhanced serum HAI antibody responses in the elderly, but mean responses were usually <2-fold [29]. Human trials have also used Qs21 (a derivative of saponin) and monophosphoryl lipid A, but results have not been reported. Finally, in a small group of volunteers, a derivative of muramyl dipeptide, the adjuvant fraction of mycobacteria, induced major reactions that appeared to have a multicomponent explanation [30]. Thus, addition of a number of newer adjuvants to IVVs has not yet led to increases in serum antibody responses comparable to those reported decades ago.

In addition, reports of enhanced antibody responses among elderly persons given thymosin α1 along with IVV suggest value from adding an immunomodulator for enhancing (or restoring) immune responses among selected populations [31].

A great variety of adjuvants and immunomodulators have been shown to enhance immune responses for various antigens.
in animal models. Despite the lack of a major enhancing effect in recent human studies, it remains possible that one or more of these immune-enhancing agents will prove of value in humans. Adjuvants could be of particular value in a pandemic circumstance either for the large number of persons who would be unprimed or as a means of conserving antigen that is available in limited quantities.

**Mucosal administration.** The need for protective levels of specific antibody in respiratory secretions for prevention of influenza virus infection was emphasized by Francis [32] >50 years ago. Current knowledge of immunoglobulin occurrences in respiratory secretions indicates that there is variation in concentrations of the different isotypes within the respiratory system. IgA antibody is dominant in nasal secretions, generally constituting ~90% of the immunoglobulin present [33]. With descent into the lower respiratory tract, the proportion of immunoglobulin that is IgG increases. Reynolds et al. [34] reported that about one-third of the immunoglobulin recovered in secretions from lower respiratory passages was IgA and two-thirds was IgG. In quantitations of specific influenza virus antibody, we found that a higher proportion of the antibody was IgG in both nasal and lower respiratory secretions [11]. It should be noted that IgG antibody in secretions is derived primarily from circulating IgG antibody, while IgA antibody is derived primarily from local lymphoid tissues [33].

Available data indicate that the airborne route is the primary means for spread of influenza [11]. When breathing is through the nose, virus is about equally likely to deposit initially in the lower or the upper respiratory passages. This probability, in combination with the apparent increased susceptibility of the lower tract, suggests that most cases of influenza are initiated in the lower tract [11]. Since the dominant immunoglobulin in secretions at this site is IgG, which is derived primarily from serum, serum IgG antibody appears to be the most important antibody for providing protection against influenza. Nevertheless, infection of the upper respiratory tract could occur initially as well as secondarily to infection in the lower tract; therefore, it is also desirable to induce IgA antibody at this site in quantities sufficient for prevention of infection.

Following the description of the mucosal immune system for secretion of IgA antibody, a number of studies of antibody responses in respiratory secretions were conducted. In the 1970s, studies indicated that the intranasal administration of IVV ensured the occurrence of antibody in nasal secretions of elderly persons and enhanced levels among seropositive persons [35–37]. A combination of parenteral and intranasal administration appeared to be optimal for inducing antibody in both serum and nasal secretions.

More recently, considerable attention has been brought to the potential of oral immunization to induce IgA antibody in

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**Figure 1.** Mean serum neutralizing (Neut) antibody (Ab) titers to A/Taiwan (H1N1) virus among persons with prevaccination titers of \( \leq 1:8 \) in hemagglutination (HA)-inhibition tests (low antibody) or \( \geq 1:16 \) (high antibody) in relation to vaccine dose and time after vaccination. From data in [17].

**Figure 2.** Frequency of persons with increase of IgA and IgG antibody to A/Taiwan (H1N1) HA in nasal wash specimens (0 and 2 weeks) in relation to vaccine dose. High and low prevaccination antibody groups in figure 1 were combined. From data in [17].
secretions at distant sites. A series of oral immunizations with IVV in enteric-coated capsules has been conducted by Waldman and colleagues [38–40]. Those studies reported that IgA antibody could be induced in nasal or salivary secretions in 50%–80% of persons despite the absence of an increase in serum antibody. This occurrence of an IgA antibody increase in secretions following oral immunization has been recently confirmed [41]. We have explored a range of doses of IVV by the oral route and also found IgA increases in nasal secretions, but frequencies were low (Cate TR, et al., unpublished data). Although incompletely explored, it seems clear that oral immunization with IVVs must be administered in very large doses or be administered with a mucosal adjuvant or other method for enhancing responses.

### Pandemic Influenza

The world has faced several pandemic threats in the time since IVVs have been available: Asian influenza in 1957, Hong Kong influenza in 1968, swine influenza in 1976, and Russian influenza in 1977. Although the US program was plagued with problems in 1976, the IVV development program provided important information on the variables influencing immune responses to vaccine [42]. A smaller program provided similar information for Russian influenza vaccines in 1977 [43]. Depending upon the uniqueness of the HA and NA, IVV for a new pandemic virus could require two doses for satisfactory immunization of substantial portions of the world’s population. For this reason, current developmental efforts that would obviate this potential problem deserve attention. Efforts such as adjuvant development that could lead to satisfactory immune responses with lower doses of antigen would decrease the burden of vaccine production. Alternatively, an increase in production capability by industry could lessen the supply problem, an item being addressed in current pandemic planning. In both instances, current approaches to improve influenza vaccines during interpandemic periods should relate to our ability to respond to the threat of pandemic influenza.

Of greater concern than vaccine supply are the data suggesting that conventional IVVs are less effective against pandemic viruses than against interpandemic epidemic viruses. A number of studies assessed protection against A/Hong Kong (H3N2) in 1968–1969. A summary of randomized, placebo-controlled studies that were identified is shown in table 3 [21, 44–47]. With the exception of 80% protection in children reported by Sugiura et al. [47], levels of protection were relatively low for the standard 200–400 chick cell agglutinating units dose. Of note are the increased efficacy rates among young adults and elderly persons when the vaccine dose was increased 10-fold [21].

Reported efficacy rates for protection against illness in children and young adults who received an IVV for Asian influenza in 1957 were generally higher (42%–77%) than those for A/Hong Kong in 1968–1969 [48, 49]. This difference perplexed Tyrrell [46], who participated in both the 1957 and 1968–1969 trials in Britain. Among the possibilities he considered for explaining the efficacy disparity were uncertainty about the “quality” of antibody, presence of anti-NA antibody, and fall in serum or secretion (or both) antibody titer between vaccination and occurrence of the epidemic. Since serum antibody at the time of the epidemic was still neutralizing and there was evidence against a fall in serum titers, he favored a decline in secretion antibody as an explanation for the difference. Vaccinations in 1957 had commenced along with the epidemic, whereas an interval of several months separated vaccinations and the epidemic of 1969; Tyrrell suggested that more antigen should be used in the vaccine to minimize this problem. Thus, IVVs can prevent pandemic influenza, but there is uncertainty about which IVV characteristics are necessary and the vaccination time frame (i.e., time between vaccination and pandemic) required to ensure optimal protection.

### Final Comments

If a pandemic influenza threat should arise in the near future, IVV would play a role and probably the major role in public health efforts to reduce its impact. Current IVVs are safe and effective for prevention of influenza and its consequences, but the protection is less than desirable because it varies among population groups and is sometimes low. Moreover, there is experience available from prior pandemics suggesting that IVVs may be even less effective in pandemic situations than in interpandemic epidemic outbreaks. Since IVV will constitute a major component of any pandemic response in the near future, improvement of current vaccines is needed for both pandemic and interpandemic epidemic influenza. There are a number of options for improvement of the protective capacity of IVVs in the near future that are deserving of effort, including increasing

<table>
<thead>
<tr>
<th>Study group, reference no.</th>
<th>Dose (CCA)</th>
<th>% infection, control</th>
<th>% efficacy*</th>
</tr>
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<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[47] 200</td>
<td>27.5</td>
<td>80</td>
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</tr>
<tr>
<td>[44] 400</td>
<td>16</td>
<td>~15</td>
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<tr>
<td>[46] 700 HA</td>
<td>26</td>
<td>27</td>
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</tr>
<tr>
<td></td>
<td>59</td>
<td>0</td>
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</tr>
<tr>
<td></td>
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<td>Adults</td>
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<tr>
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<td>34</td>
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</tr>
<tr>
<td></td>
<td>9.5</td>
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<tr>
<td>[21] 300</td>
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<tr>
<td></td>
<td>3000</td>
<td>9.5</td>
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</table>

NOTE. CCA = chick cell agglutinating units; HA = hemagglutinin units.
* Efficacy for serologic evidence of infection.
References