Live Victoria/75-ts-1[E] Influenza A Virus Vaccines in Adult Volunteers: Role of Hemagglutinin Immunity in Protection Against Illness and Infection Caused by Influenza A Virus

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To explore the relationship between neuraminidase immunity and the degree of attenuation of live influenza A virus vaccines, a comparative evaluation of three Victoria/75-ts-1[E] (Vic/75-ts-1[E]) recombinant viruses in serum hemagglutination-inhibiting-negative (titer, ≤1:8) adult volunteers was performed. These three ts-1[E] viruses had a similar restriction of replication at 38°C in vitro, and each possessed the two attenuating genes of the ts-1[E] donor strain (13). However, Vic/75-ts-1[E] recombinants 81 and 113 possessed both Vic/75 hemagglutinin (H375) and Vic/75 neuraminidase (N275), whereas Vic/75-ts-1[E] recombinant 67 had Vic/75 hemagglutinin but the N265 neuraminidase. Vic/75-ts-1[E] recombinant 67 was significantly more attenuated than Vic/75-ts-1[E] recombinants 81 and 113 in that fewer local and systemic signs and symptoms of illness were observed in those volunteers who received clone 67. These findings were consistent with our previous observations which suggested that the following two factors contribute to the attenuation of ts-1[E] vaccine strains in adults: (i) the attenuating effect of the two ts-1[E] genes and (ii) the neuraminidase immunity of the host. Vic/75-ts-1[E] recombinant clone 67 vaccinees developed an immunological response to the H375 hemagglutinin in the absence of a response to the N275 neuraminidase. To assess the role of anti-hemagglutinin immunity induced by an attenuated live virus vaccine plays in resistance to influenza A virus, vaccinees who received recombinant 67 were challenged with Vic/75 wild-type virus, and their responses were compared with those of Vic/75-ts-1[E] vaccinees who received recombinant 81 or 113. Each of the three groups of ts-1[E] vaccinees was significantly protected against illness induced by wild-type virus infection, although resistance was not complete. However, the clone 67 vaccinees were protected less against infection. The infection-permissive resistance induced by clone 67 resembled that previously described for inactivated neuraminidase-specific vaccines. These results suggested that a ts-1[E] recombinant that possessed the hemagglutinin of a new pandemic variant, the neuraminidase of the preceding subtype, and the two ts-1[E] ts genes would be satisfactorily attenuated for children and adults with neuraminidase immunity and could induce resistance to illness caused by the new pandemic wild-type influenza A virus.

The influenza A/Hong Kong/68-ts-1[E] (H396N265) virus and its Udorn/72 and Georgia/74 recombinants were satisfactorily attenuated in adult volunteers (7, 8, 14, 15). In addition, these candidate vaccines were genetically stable in adult volunteers and provided protection against homologous wild-type virus challenge (7, 15). In these studies, volunteers lacked serum hemagglutination-inhibiting (HAI) antibody (titer, ≤1:8), but possessed serum neuraminidase-inhibiting (NI) antibody to the candidate vaccine virus.

When the Hong Kong/68- and Udorn/72-ts-1[E] viruses were administered to children who lacked both serum HAI and serum NI antibodies, febrile responses were observed, and virus which had lost the temperature-sensitive (ts) phenotype was recovered (3, 17). These studies suggested that neuraminidase immunity could ameliorate the clinical response to the live influ-
enza A virus vaccine just as it has been shown to do for wild-type virus infection (10). Subsequently, experience with influenza A/Victoria/3/75-ts-1[E] (A/Vic/3/75-ts-1[E]) recombinant viruses 81 and 113 in adults revealed that 12% developed fever or systemic reactions (11). In these studies, the adult volunteers lacked both serum HAI antibody (titer, ≤1:8) and serum NI antibody (titer, ≤1:4). The occurrence of febrile responses in these vaccinees was attributed to their lack of both HAI and NI immunity.

To explore further the relationship of NI antibody to the degree of attenuation of ts influenza A viruses, we performed a comparative evaluation of three Vic/75-ts-1[E] recombinants. Two of these, Vic/75-ts-1[E] clones 81 and 113, possessed the Vic/75 neuraminidase (N275), and the third, clone 67, possessed the N2α, antigen present on the Hong Kong/68-ts-1[E] parent virus (9). Since the volunteers tested possessed serum NI antibody for the N2α antigen but not the N275 antigen, it was possible to evaluate ts-1[E] recombinants with the same ts lesions and ts phenotype (38°C shutoff for plaque formation) in serum HAI antibody-negative individuals with or without neuraminidase immunity. Also, vaccinees who received the Vic/75-ts-1[E] clone 67 (H375N2α) virus developed serum HAI antibody against the Vic/75 hemagglutinin but not against the Vic/75 neuraminidase. In the present study challenge of these vaccinees with Vic/75 wild-type virus permitted an evaluation of the role of anti-hemagglutinin antibody in protection against influenza A virus infection and illness.

MATERIALS AND METHODS

Viruses. The wild-type A/Vic/3/75 virus was isolated in specific pathogen-free eggs as described previously (9). The virus suspension administered to volunteers was uncloned and had undergone two passages in specific pathogen-free eggs (11).

The production of the A/Vic/3/75-ts-1[E] recombinant viruses by mating the A/Hong Kong/68-ts-1[E] attenuated donor virus and the A/Vic/3/75 wild-type virus has been described previously (9). The antigenic and genetic properties of these Vic/75-ts-1[E] recombinants have also been described (9) and are summarized in Table 1. The procedures for cloning, production of virus suspensions for administration to volunteers, and safety testing of these suspensions have been described (11).

Evaluation of the viruses in volunteers. Healthy volunteers, 18 to 60 years old, who possessed a serum HAI titer of ≤1:8 to the A/Vic/3/75 (H3N2) virus participated in the study after informed consent was obtained. The methods for the isolation of volunteers, administration of virus, observation of clinical response, isolation of virus in rhesus monkey kidney culture, quantitation of virus, characterization of isolates for the retention of the ts property, and serological tests that were used at the University of Rochester and at the Clinical Research Center for Vaccine Development at the University of Maryland have been described previously (11).

The clinical evaluations of Vic/75-ts-1[E] recombinant clone 81 and Vic/75 wild-type viruses have been described and are included in the present study for comparison (Tables 2 and 3) (11). Clinical evaluations of 12 of the 22 volunteers who received Vic/75-ts-1[E] clone 113 have also been described (Table 2) (11). Published data derived from Vic/75 wild-type challenge of nine vaccinees who received Vic/75-ts-1[E] clone 81 are also included (Table 3) (11). Vic/75-ts-1[E] clone 67 had not been tested previously.

Since ts-1[E] vaccine strains had been shown previously to be nontransmissible in humans (11, 15), a double-blind evaluation of clones 113 and 67 was performed at the University of Rochester in volunteers who were housed together in the same isolation facility. This double-blind study permitted not only unbiased clinical evaluation but also an accurate comparison of virus replication since the same lot of primary rhesus monkey tissue was used to isolate virus and to quantitate the amount of virus present in the nasopharyngeal wash specimens. This is important because lots of this primary tissue can vary up to 100-fold in sensitivity to the same influenza A virus (Murphy, unpublished data). Thus, a meaningful comparison of level of virus replication was achieved by using one lot of tissue for this study. To quantitate more precisely the symptoms produced by these two ts-1[E] viruses in the double-blind study, each sign and each symptom were given a severity score of 0, 1, 2, or 3 so that total sign and symptom scores could be calculated for each volunteer (4). Signs and symptoms were also classified as local or systemic, and the resulting illness categories are presented in Table 2. Oral temperatures were recorded four times each day.

RESULTS

Response of volunteers to Vic/75-ts-1[E] wild-type virus. The data presented in Table

| Table 1. Antigenic and genetic properties of viruses employed in this study |
|-----------------------------|---------------------|---------------------|---------------------|
| Virus                      | Shut-off temp. (°C) | ts lesion on gene: | Hemagglutinin subtype | Neuraminidase subtype |
| Vic/75-ts-1[E] recombinants |                     | P3 NP              |                     |                     |
| Clone 67                   | 38                  | Yes Yes            | H375               | N2αβ               |
| Clone 113                  | 38                  | Yes Yes            | H375               | N2αβ               |
| Clone 81                   | 38                  | Yes Yes            | H375               | N2αβ               |
| Vic/75 wild type           | >39                 | No No              | H375               | N2αβ               |

* Shutoff temperature is defined as the lowest temperature at which a 100-fold reduction of plaque formation occurs compared with the titer at the permissive temperature (34°C). β Clone 67 derived the N2α neuraminidase from the Hong Kong/68-ts-1[E] (H3ααN2αα) (13) parent.
<p>| Table 2. Response of seronegative volunteers to A/Vic/3/75-ts-1[E] or wild-type virus |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Vic/75 virus administered</th>
<th>N2 neuraminidase</th>
<th>Dose (log&lt;sub&gt;10&lt;/sub&gt; TCID&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. tested</th>
<th>% infected&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% who shed</th>
<th>Avg duration (days)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Peak mean log&lt;sub&gt;10&lt;/sub&gt; titer</th>
<th>Reciprocal of mean log&lt;sub&gt;10&lt;/sub&gt; titer</th>
<th>% with fourfold or greater rise in antibody titer</th>
<th>Reciprocal of mean log&lt;sub&gt;10&lt;/sub&gt; titer to homologous N2 antigen</th>
<th>% with 1.5 log&lt;sub&gt;10&lt;/sub&gt; or greater rise in antibody titer</th>
<th>Febrile (≥37.8°C) and/or systemic symptoms</th>
<th>Upper respiratory tract</th>
<th>Any&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Double-blind study</td>
<td>N2&lt;sub&gt;60&lt;/sub&gt;</td>
<td>7.2</td>
<td>10</td>
<td>60</td>
<td>20</td>
<td>0.5 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.6 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.9 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.6 ± 0.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>50</td>
<td>6.2 ± 0.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.1 ± 0.2</td>
<td>20</td>
<td>10</td>
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<tr>
<td>ts-1[E] clone 67</td>
<td>N2&lt;sub&gt;70&lt;/sub&gt;</td>
<td>6.5</td>
<td>10</td>
<td>80</td>
<td>30</td>
<td>1.2 ± 0.7</td>
<td>1.3 ± 0.5</td>
<td>2.4 ± 0.2</td>
<td>4.2 ± 0.4</td>
<td>60</td>
<td>≥0.5</td>
<td>0.1 ± 0.2</td>
<td>20</td>
<td>20</td>
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<td>Cumulative data</td>
<td>N2&lt;sub&gt;60&lt;/sub&gt;</td>
<td>7.2</td>
<td>28</td>
<td>75</td>
<td>56</td>
<td>1.7 ± 0.3</td>
<td>1.4 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>4.2 ± 0.3</td>
<td>50</td>
<td>6.1 ± 0.3</td>
<td>6.9 ± 0.3</td>
<td>21</td>
<td>4</td>
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<tr>
<td>ts-1[E] clone 113</td>
<td>N2&lt;sub&gt;70&lt;/sub&gt;</td>
<td>6.5</td>
<td>22</td>
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<td>7.2</td>
<td>52</td>
<td>84</td>
<td>67</td>
<td>3.0 ± 0.3</td>
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<td>2.4 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>60</td>
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<td>1.0 ± 0.2</td>
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<td>N2&lt;sub&gt;70&lt;/sub&gt;</td>
<td>5.2</td>
<td>19</td>
<td>100</td>
<td>95</td>
<td>4.8 ± 0.4</td>
<td>3.5 ± 0.4</td>
<td>2.1 ± 0.2</td>
<td>4.4 ± 0.4</td>
<td>67</td>
<td>0.1 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>44</td>
<td>53</td>
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</table>

<sup>a</sup> TCID<sub>50</sub>, 50% tissue culture infective dose.
<sup>b</sup> Evidence of virus shedding or a rise in serum antibody or both.
<sup>c</sup> Each vaccinee was tested daily for 7 days; each volunteer who received wild-type virus was tested daily for 10 days. Only data from infected volunteers are included.
<sup>d</sup> The amount of virus in the nasopharyngeal wash specimens from each volunteer was determined, and the maximum amount shed by each volunteer was averaged. The specimens from only 15 of the 52 volunteers who received clone 81 were analyzed. Only data from infected volunteers are included.
<sup>e</sup> HAI antibody titers were tested by using H<sub>20</sub>N2<sub>70</sub> virus as test antigen for virus possessing the N2<sub>70</sub> neuraminidase and H<sub>70</sub>N2<sub>70</sub> for viruses possessing the N2<sub>70</sub> neuraminidase.
<sup>f</sup> Upper respiratory tract illness is defined as an illness which is observed by two physicians on 2 consecutive days and which consists of either or both of the following: (i) pharyngitis, the occurrence of pharyngeal erythema and discomfort, and (ii) rhinitis, the development of rhinorrhea. Systemic illness is defined as the occurrence of myalgias or chills and sweats.
<sup>a</sup> For the cumulative data, P < 0.05 in comparisons of clones 67 and 113 and clones 67 and 81.
<sup>a</sup> Mean ± standard error.
2 summarize the clinical, virological, and serological responses of serum HAI-negative adult volunteers to the Vic/75-ts-1[E] recombinants and wild-type virus. Data derived from both the double-blind study and the cumulative analysis are presented. In the double-blind study and in the cumulative analysis all groups of volunteers had similar levels of pre-inoculation serum HAI antibody. However, the pre-inoculation serum NI antibody titers in vaccinees who received Vic/75-ts-1[E] recombinant clone 67 were higher than the serum NI antibody titers of volunteers who received either a ts or a wild-type virus bearing the N275 antigen (Table 2). In the double-blind study, 5 of 10 clone 113 vaccinees developed illness; 3 were upper respiratory tract illnesses, and 2 were predominantly systemic. One of the latter two volunteers had a fever which peaked at 100.3°F (37.9°C) and lasted for 8 h. In contrast, only one vaccinee who received clone 67 developed mild upper respiratory tract illness, which lasted for 24 h, and none developed fever or other systemic manifestations of illness. Although the differences in the number of vaccinees who became ill in the double-blind study did not achieve statistical significance, a comparison of the mean illness scores, which is a measure of severity and duration of illness, indicated that the clone 67 vaccinees had significantly less illness than the clone 113 vaccinees (P < 0.05; Wilcoxin test). Moreover, when the cumulative data are considered (Table 2), the number of clone 67 vaccinees who became ill was significantly less than the number of clone 113 or 81 vaccinees who became ill. All three ts-1[E] vaccines were attenuated compared with wild-type virus.

In the double-blind study, the vaccinees who were infected by clone 67 shed less virus for a shorter duration than did clone 113 vaccinees, although these differences did not achieve statistical significance. In the cumulative data the three ts-1[E] recombinants were shed in reduced amount and for shorter duration compared with wild-type virus. All 36 clone 67 isolates, all 34 clone 113 isolates, and 121 of 123 clone 81 isolates retained the ts phenotype.

The serum HAI response of each group of ts-1[E] vaccinees was comparable to that of volunteers receiving wild-type virus. Whereas approximately 20% of vaccinees had a serum NI antibody rise to the homologous neuraminidase antigen, none of the clone 67 vaccinees had a rise in the N275 neuraminidase.

**Response to challenge with wild-type A/Vic/3/75 virus.** The vaccinees who received clone 67 and who developed an antibody response to the hemagglutinin antigen of the Vic/
75 wild-type virus but not to its neuraminidase were challenged with this wild-type virus to assess the contribution of hemagglutinin immunity to resistance (Table 3). The challenge data from vaccinees who received clones 113 and 81 were combined to permit statistical comparison with the data obtained from clone 67 vaccinees who were challenged. Combining the data in this manner appeared justified because the clinical and immunological responses to the clone 81 and 113 recombinant viruses were statistically indistinguishable (Table 2) and because these viruses were antigenically and phenotypically identical (9).

Both groups of ts-1[E] vaccinees resisted illness caused by wild-type virus to a comparable degree (13 versus 23%). However, resistance was incomplete in that local and systemic manifestation of illness occurred in vaccinees who received either H375N265 clone 67 or H375N275, clone 81 or 113. In contrast to protection against illness, resistance to infection with the Vic/75 wild-type virus was not identical for both groups, since vaccinees who received recombinant clone 81 or 113 were better protected than vaccinees who received recombinant clone 67 (33 versus 85%; \( P < 0.02 \)).

**DISCUSSION**

Although all three of these ts-1[E] recombinant viruses were clinically attenuated, Vic/75-ts-1[E] recombinant clone 67 was significantly more attenuated when administered to adult volunteers than were Vic/75-ts-1[E] recombinant clones 81 and 113 in that fewer local and systemic signs and symptoms of illness were observed in the volunteers who received clone 67. This difference was observed despite the fact that all three of these viruses exhibited a similar level of restriction of replication in vitro and in hamster lung with respect to wild-type virus (9) and that each possessed the attenuating P3 and nucleoprotein ts genes (5, 9). The major identifiable immunological difference among the serum HAI-negative (titer, \( \leq 1:8 \)) vaccinees was the presence of antibody to the N265 neuraminidase antigen of the vaccine virus in those volunteers who received clone 67 and the lack of antibody to the N275 antigen in individuals who received clone 81 or 113. Since antibody to the neuraminidase surface glycoprotein has been shown to provide resistance to influenza A virus illness in animals and in humans, it was not surprising to observe that the vaccinees with higher levels of neuraminidase immunity experienced less illness than those with lower levels (1, 6, 10, 16). The present findings are consistent with our previous suggestion that the following two factors contribute to the attenuation of ts-1[E] vaccine strains in serum HAI-negative adults who possess NI immunity: (i) the attenuating effect of the two ts-1[E] ts genes and (ii) the neuraminidase immunity of the host (3). It is possible that genes not identified by our serological or genetic analysis (i.e., polymerases P1 and P2, matrix protein, M, and nonstructural protein [12, 13]) may also have contributed to the differences in clinical response to clone 67 and clones 81 and 113. A detailed examination of this question suggesting that these genes were not responsible for the observed differences is presented in another paper (5). In any case, satisfactory attenuation of ts-1[E] recombinants in adults with NI immunity suggests that such viruses may be acceptable for immunization against interpandemic influenza when a major drift in the neuraminidase antigen does not occur.

Since the two ts-1[E] ts genes do not provide sufficient attenuation for vaccinees who lack neuraminidase immunity, it is not likely that these mutant genes would satisfactorily attenuate a wild-type virus at the time of a major antigenic shift in both surface antigens. However, a ts-1[E] recombinant with the hemagglutinin of the new pandemic variant, the neuraminidase of a preceding subtype, and the two ts-1[E] ts genes would probably be satisfactorily attenuated for children and adults with neuraminidase immunity. Such a vaccine strain would induce only hemagglutinin-specific immunity. The capacity of hemagglutinin-specific immunity induced by an attenuated vaccine strain to provide resistance to influenza A wild-type virus in humans has not been defined previously. In the present study we were able to investigate this question because the clone 67 (H375N265) vaccinees developed an immunological response to the H375 neuraminidase in the absence of a response to the N275 neuraminidase. Such vaccinees were protected against illness caused by Vic/75 wild-type challenge virus, and this level of resistance was comparable to that induced by Vic/75-ts-1[E] (H375N265) strains (clones 81 and 113). However, the clone 67 vaccinees were less protected against infection than were the latter group. The infection-permissive resistance to illness induced by clone 67 resembled the resistance previously described for inactivated neuraminidase-specific vaccine (2). It is possible that levels of hemagglutinin-specific immunity higher than those induced by the clone 67 ts-1[E] virus might also protect against infection, but this remains to be demonstrated. In any case, the present study indicates that it is possible to protect against influenza A virus illness by hemagglutinin-specific immunity induced by a live attenuated virus. The potential for this
type of immunization in the prevention of pandemic influenza illness remains to be established.

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LITERATURE CITED