Dose-Related Safety and Immunogenicity of Baculovirus-Expressed Trivalent Influenza Vaccine: A Double-Blind, Controlled Trial in Adult Patients with Non-Hodgkin B Cell Lymphoma

Amar Safdar,1 M. Alma Rodriguez,2 Luis E. Fayad,2 Gilhen H. Rodriguez,1 Barbara Pro,2 Michael Wang,1 Jorge E. Romaguer,3 Andre H. Goy,2 Fredrick B. Hagemeister,2 Peter McLaughlin,2 Gerald P. Bodey,1 Larry W. Kwak,7 Issam I. Raad,1 and Robert B. Couch2

Departments of 1Infectious Diseases, Infection Control, and Employee Health and of 2Lymphoma and Myeloma, University of Texas M. D. Anderson Cancer Center, and 3Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas

In 27 patients randomized to receive commercial trivalent influenza vaccine (TIV) containing 15 µg of the hemagglutinin (HA) of influenza A (H3N2 and H1N1) and B virus or a recombinant vaccine (rHAO) containing 15, 45, or 135 µg of each HA, reactogenicity was minor. Among patients with similar prevaccination titers, 40% given 45 µg and 60% given 135 µg of rHAO developed an increase in influenza A/H3 neutralizing antibody levels; there were no increases in 4 given TIV. For each vaccine, the highest frequencies of increases in neutralizing antibody levels and the highest mean titers occurred in those given the 135-µg vaccine.

Influenza can be a serious infection in patients with hematologic malignancy [1, 2]. The current Centers for Disease Control and Prevention recommendation for patients with cancer to receive standard influenza vaccine (SV) [3] is complicated by the fact that most patients with hematologic malignancy receiving antineoplastic therapy fail to exhibit protective immune responses; and, despite receiving appropriate vaccination, many patients may remain vulnerable to a debilitating influenza infection [4–6].

A number of measures have been explored for improving responses to influenza vaccine, including a second vaccine dose to boost suboptimal responses that followed a single vaccination. At our institution, a second dose of SV was associated with a nearly 30% increase in responses among patients with non-Hodgkin B cell lymphoma (NHL) [6]. This benefit with 2 sequential vaccine doses, however, was not seen in a recent European study in patients with hematologic cancer [5]. Another approach for improving the response to vaccination might be to increase the antigen dose. Since the introduction of influenza vaccines, increasing the dosage of the hemagglutinin (HA) antigen has been shown repeatedly to increase serum antibody responses in a number of populations, but there has not been a dose-response evaluation in lymphoma patients [7, 8]. Recent studies of increasing dosage have employed purified and recombinant DNA (rDNA)–produced HA vaccines as a means of increasing the antibody responses without significantly increasing reactogenicity [9, 10]. A recent trial of an rDNA-produced HA vaccine in the elderly with the same vaccine used in this study demonstrated significant increase in antibody responses to the A/H3 component with increasing dosage, with only a mild increase in local reactogenicity from the highest dosage [10]. The present study sought to determine whether increasing the dosages of this same vaccine in patients with NHL would also improve vaccine responses.

Patients, materials, and methods. This study was approved by the institutional review boards of the M. D. Anderson Cancer Center (MDACC) and Baylor College of Medicine, Houston, Texas; written, informed consent was obtained from all patients. Patients were recruited from the adult lymphoma clinic at the MDACC between 23 August and 23 October 2004. They were randomized to receive either SV or 1 of 3 increasing dosages of investigational recombinant vaccine. The vaccine was prepared at the M. D. Anderson Cancer Center following current good manufacturing practice guidelines as rDNA-produced HA vaccine in the elderly with the same vaccine used in this study demonstrated significant increase in antibody responses to the A/H3 component with increasing dosage, with only a mild increase in local reactogenicity from the highest dosage [10]. The present study sought to determine whether increasing the dosages of this same vaccine in patients with NHL would also improve vaccine responses.

Patients were eligible if they had biopsy-proven NHL. The Ann Arbor stage was determined after review of all clinical, laboratory, histologic, and radiographic information. The histologic classification was based on the Revised European-American Classification of Lymphoid Neoplasms [11]. Only patients who had not received chemotherapy in the 3 months before...
enrollment were considered. They were ineligible if they were allergic to influenza vaccine or egg products or had undergone a splenectomy. Individuals who had received rituximab and parenteral immunoglobulin within the 6-month period before enrollment were also excluded from the study. Patients who had received systemic corticosteroids, other investigational vaccine, or antineoplastic medications in the 4 weeks before enrollment were also excluded. Because of eligibility criteria and the inconvenience of repeated visits, only 27 subjects agreed to participate during the enrollment period.

The SV used was commercially available split-virus TIV from a single lot containing 15 μg/0.5 mL each of the HA of influenza A/Panama/2007/99 (H3N2), A/New Caledonia/20/99 (H1N1), and B/Hong Kong/330/2004 (Sanofi Pasteur). The recombinant HA protein vaccine consisted of HA expressed in insect (SF9) cells by recombinant baculovirus. The HA genes of the 3 influenza viruses contained in the vaccine (A/Panama/2007/99 H3N2, A/New Caledonia/20/99 H1N1, and B/Hong Kong/330/2001) were independently cloned into the plasmid baculovirus expression vector pPSC12. The vector contained the AcNPV baculovirus polyhedron promoter, the baculovirus 61K signal peptide, and flanking baculovirus DNA derived from the EcoRII fragment of AcNPV. After confirmation of the correct sequences, the DNA sequences were inserted into AcNPV by homologous recombination. Recombinant virus containing the respective HA genes were then used to express the HA s in the high-yield SF9-derived insect cell line.

The proteins were purified to >95% purity as assessed by protein gel electrophoresis, and the final concentration was determined by modified Lowry assay. The vaccine was supplied at a final concentration of either 15 μg, 45 μg, or 135 μg of each HA per 0.5-mL dose (45-μg, 135-μg, or 405-μg total doses of rHAO).

After study initiation, assays of the rHAO vaccines by single radial immunodiffusion, the basis for SV quantitation, revealed that the dose of A/Panama/2007/99 rHAO (H3) by single radial immunodiffusion (SRID) was equivalent to that determined by the Lowry method. The amount of A/New Caledonia/20/99 rHAO (H1) by SRID was only 30% of that determined by Lowry, and the amount of the B/Hong Kong/330/2001 rHAO by SRID was 80% of that determined by Lowry. For purposes of data presentation, the dosage groups are referred to as originally intended (i.e., as the 15-μg, 45-μg, and 135-μg groups).

Tests for hemagglutination inhibition (HAI) and neutralizing antibodies in serum samples were performed using methods described elsewhere [12, 13]. For the HAI antibody test, concentrations of reagents were altered, and an erythrocyte-ad sorption step was added to permit a starting dilution of 1:4. Test viruses for HAI assays were A/Panama/2007/99 (H3N2), A/New Caledonia/20/99 (H1N1), and B/Hong Kong/330/2004. The same viruses were used in neutralizing tests for influenza B and influenza A/H1N1 antibody, but A/Moscow/10/99 (H3N2) (antigenically similar to A/Panama/H3N2 virus) was used in influenza A/H3N2 tests.

A 4-fold increase in HAI or neutralizing antibody titers between before and 4 weeks after vaccination was considered to

---

### Table 1. Nos. of patients with 4-fold or greater increases in titers of serum hemagglutination inhibiting (HAI) and neutralizing (neut) antibodies against influenza A (H3 and H1) or B virus between before and 4 weeks after vaccination.

<table>
<thead>
<tr>
<th>Vaccine, dose</th>
<th>A/H3</th>
<th>A/H1</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAI</td>
<td>Neut</td>
<td>Either</td>
</tr>
<tr>
<td>Standard, 15 μg (n = 6)</td>
<td>2</td>
<td>1</td>
<td>2 (33)</td>
</tr>
<tr>
<td>rHAO, 15 μg (n = 9)</td>
<td>3</td>
<td>4</td>
<td>4 (44)</td>
</tr>
<tr>
<td>rHAO, 45 μg (n = 6)</td>
<td>1</td>
<td>3</td>
<td>3 (50)</td>
</tr>
<tr>
<td>rHAO, 135 μg (n = 6)</td>
<td>3</td>
<td>3</td>
<td>3 (50)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients.

---

### Table 2. Serum neutralizing antibody responses to influenza A (H3 and H1) or B virus in subjects with similar prevaccination titers.

<table>
<thead>
<tr>
<th>Vaccine, dose</th>
<th>A/H3</th>
<th>A/H1</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. with increase/total no. (%)</td>
<td>PoV/PreV (fold increase)</td>
<td>No. with increase/total no. (%)</td>
</tr>
<tr>
<td>Standard, 15 μg</td>
<td>0/4 (0)</td>
<td>97.0/45.3 (2.1)</td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>rHAO, 15 μg</td>
<td>1/4 (25)</td>
<td>238.9/78.8 (3.0)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>rHAO, 45 μg</td>
<td>2/5 (40)</td>
<td>157.6/45.3 (2.5)</td>
<td>1/4 (25)</td>
</tr>
<tr>
<td>rHAO, 135 μg</td>
<td>3/5 (60)</td>
<td>415.9/78.8 (5.3)</td>
<td>2/3 (67)</td>
</tr>
</tbody>
</table>

NOTE. PoV, highest geometric mean titer (GMT) 4 or 8 weeks after vaccination; PreV, prevaccination GMT.

* No. of patients with a 4-fold or greater increase/total no. of patients.
be a response to a vaccine. Geometric mean titers for each vaccine-dose group were calculated.

A diary card was provided to patients for self-documentation of adverse reactions that included soreness, tenderness, erythema, or swelling at the vaccination site; malaise, myalgia, feeling feverish, and actual fever were also recorded. Safety follow-up visits were at 7–10 days, a mean ± SD of 28 ± 4 days, and a mean ± SD of 56 ± 4 days after receiving vaccination. A final safety assessment was performed by contacting patients via telephone a mean ± SD of 180 ± 14 days after vaccination.

**Results.** Fifteen subjects were men, and 12 were women; the average age was 55 years. Fourteen patients (52%) had diffuse-large B cell lymphoma; 4 (15%) each had follicular small cleaved cell and follicular lymphoma. T cell–rich B cell lymphoma, small lymphocytic lymphoma, marginal B cell lymphoma, and mantle cell and Burkitt cell lymphoma were present in 1 patient (4%) each.

Earlier antineoplastic therapy had consisted of cyclophosphamide, doxorubicin, vincristin, and prednisone (CHOP) in 13 patients (48%); 10 (37%) of these also received concurrent rituximab (monoclonal antibody with high affinity for CD20-expressing cells). Five patients (19%) had received hyper cyclophosphamide, mesna, doxorubicin, and vincristine plus rituximab. Three had been treated with rituximab plus fludarabine, mitoxantrone, and dexamethasone. Two patients (7%) each had received CHOP–etoposide, cisplatin, cytarabine, and methylprednisolone–mitoxantrone, vincristine, procarbazine, and prednisone; in 1 patient, rituximab had been added. One patient each had received rituximab plus granulocyte-macrophage colony-stimulating factor and bortezomib (Valcane; a proteasome inhibitor). Two patients (7%) had not received antineoplastic therapy.

The number of patients with an increase in serum antibody titer in relation to vaccine and dose is shown in table 1. There was a trend toward an increased antibody response frequency in the higher rHAO dose groups. The response frequencies were higher for A/H3 and A/H1 than for SV but not for influenza B; however, none of the differences were statistically significant.

Shown in table 2 are neutralizing antibody increases for persons with similar prevaccination titers. Two groups (50%) of 5 patients who had received 45 μg and 3 (60%) of 5 given 135 μg of rHAO exhibited an increase in influenza A/H3 neutralizing antibody titer, compared with no increases in 4 patients in whom SV was given and only 1 (25%) of 4 for the 15-μg group. Moreover, the mean titers increased with increasing dosage. For influenza A/H1, 2 (40%) of 5 patients who received SV and 2 (67%) of 3 who received 135 μg of rHAO developed a response. Neutralizing antibody responses for influenza B were not different in either recipients of SV (40%) or those who received 135 μg of rHAO (50%). Also, there was no clear dose response for the A/H1 and B antigens. However, the group with the highest antibody response frequency and the highest mean titer for each of the 3 viruses was the highest dose group (135 μg). Nonetheless, none of the differences were statistically significant.

All patients were followed for 6 months after vaccination, and no serious vaccine-related adverse reactions were noted. One patient given the 135-μg rHAO vaccine reported an acute respiratory illness without fever 3 days after vaccination that resolved in 14 days. Another patient given the 15-μg rHAO vaccine reported fever with malaise and myalgias for 3 days after immunization. Two patients given the 45-μg rHAO vaccine reported moderate malaise and myalgias for 1–3 days. Six other patients reported mild redness, tenderness, malaise, and/or myalgias after vaccination; all 4 vaccines were represented.

**Discussion.** In this randomized dose-escalation study, increasing concentrations of recombinant vaccine were associated with an increase in serum antibody responses against influenza A. Although the numbers were small, the highest dose given, 135 μg of each antigen, induced the highest frequency of responses for all 3 viruses. In an earlier study among elderly persons with the same vaccines, a significant increase in HAI antibody response was seen with increasing dosage for the A/H3N2 component [10]. The recombinant vaccine was safe, and no serious adverse reactions occurred in our patients with NHL. The results of this pilot study are consistent with our hypothesis that impaired antibody responses to influenza vaccine among patients with B cell lymphoma may be improved by administering higher vaccine doses. Interestingly, this benefit appeared to be greater for levels of neutralizing antibody against influenza A than against influenza B. Responses to increasing doses of influenza B vaccine have been less apparent than for influenza A in other studies [14].

Various strategies to improve vaccine responses have yielded inconsistent results in patients with underlying cancer [5, 6, 15]. In addition to increased dosages and number of doses, another possible strategy for enhancing postvaccination immune responses in immunocompromised patients may include use of bioadjuvants. Further studies are needed to determine the value of an approach that utilizes 1 or more methods to improve vaccine response in immunosuppressed patients with hematologic malignancy. The genetically engineered recombinant vaccine used in this study has the potential advantage of permitting increased dosages and sequential booster doses of cancer patients without significant reactogenicity. Further studies in cancer patients are planned.

**Acknowledgments**

We thank the Protein Sciences Corporation, Meriden, Connecticut, for provision of rHAO vaccine; Joyce Brown (study coordinator) and Celsa Tajonera of the Viral Respiratory Pathogen Research Unit clinical staff; Diane Nino and technical staff, for performance of laboratory assays; and the National Institute of Allergy and Infectious Diseases Influenza Team (Soni Kim, Jean Hu-Primmer, Lydia Falk, and Linda Lambert).
References