Impaired Serum Antibody Response to Inactivated Influenza A and B Vaccine in Renal Transplant Recipients

H. GRANT STIVER,* PATRICIA GRAVES, GORDON MEIKLEJOHN, GERHARD SCHRÖTER, AND THEODORE C. EICKHOFF

Division of Infectious Diseases, Department of Medicine, University of Colorado Medical Center, Denver, Colorado, 80220

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Before and after vaccination with a commercial inactivated influenza vaccine containing A/Aichi/2/68 (H3N2) and B/Massachusetts/1/71 antigens, the serum hemagglutination inhibition antibody titers to homologous and heterologous strains of A and B influenza viruses were measured in 45 renal transplant patients and 66 healthy controls (62 for the B strains). At least a fourfold titer rise to the homologous A strain occurred in 14 of 45 transplant patients (31%) versus 37 of 66 controls (56%). Fourfold or greater heterologous A rises occurred in only 8 of 45 transplant patients (18%) compared with 40 of 60 controls (61%). In both the homologous and heterologous B responses, at least fourfold hemagglutination inhibition titer rises were seen in significantly fewer transplant patients than control subjects. In the transplant group, no correlation was observed between degree of antibody response and age, previous influenza vaccination, percentage of patients initially seronegative, time since transplantation, dose of immunosuppressive drugs, level of renal function, or nature of original renal disease.

Epidemics of influenza A have been associated with excess mortality and morbidity in compromised hosts and the elderly (3). Recently, acute rejection of transplanted kidneys during influenza A outbreaks has been reported (1), indicating that transplant recipients may fall into the "high-risk" group. It follows that they may benefit from the protection provided by influenza vaccine. The following study was designed to assess both the homologous and heterologous humoral antibody response to influenza vaccine in a group of renal transplant patients.

MATERIALS AND METHODS

Study population. The study population consisted of two groups: (i) a group of 45 renal transplant recipients cared for under the University of Colorado transplant program, and (ii), a control group of 66 freshman medical students enrolled at the University of Colorado Medical School. The transplant patients were receiving immunosuppressive drugs, usually prednisone and azathoprine (four patients were receiving prednisone plus cyclophosphamide). None of the control population were receiving immunosuppressive drugs. The mean age of the transplant group was 28 years (range, 6 to 46 years), and the mean age of the control group was 23 years (range, 21 to 38 years).

HL-A tissue typing had been performed previously on the transplant patients. Tests were performed by Yosh Arai in the Research Laboratories, Veterans Administration Hospital, Denver, Colo., by the microdroplet lymphocyte cytotoxicity test (7) with tissue-typing plates supplied by P. I. Terasaki, UCLA School of Medicine, and by the National Institutes of Health, Bethesda, Md. Tissue types were analyzed in retrospect, and results were available for only 30 of the 45 transplant patients who received influenza vaccine.

Vaccination procedures. In October and November 1972, each individual in the study groups received 0.5 ml of bivalent ether-split aqueous inactivated influenza vaccine (Fluogen, Parke, Davis Co.) containing 700 chicken cell-agglutinating units of A/Aichi/2/68/X.31 (H3N2) and 300 chicken cell-agglutinating units of B/Massachusetts/1/71 antigen intramuscularly. Twelve transplant patients had received influenza vaccine within the previous 3 years. None of the controls had received influenza vaccine before. Before vaccination and from 4 to 6 weeks after, blood samples were drawn and the paired sera were tested to determine the antibody response.

Determination of antibody response. Paired sera were tested by the hemagglutination inhibition test with chicken erythrocytes, using the microtiter method with microtiter and macrotiter virus controls (10). The sera were tested against virus strains homologous to the vaccine antigens, A/Hong Kong/68/Hung Kong/
8/68 (H3N2) and B/Massachusetts/8/66, as well as to viruses heterologous to the vaccine antigens, A/Denver/1/72 (H3N2), which resembles the A/England/42/72 (H3N2) strain, and B/452/73, a strain similar to B/Hong Kong/5/72. All serum pairs were inactivated at 56°C and treated with receptor-destroying enzyme according to the Center for Disease Control procedure. Each batch of enzyme was checked against sera known to contain high levels of nonspecific inhibitors (10). Statistical analysis was done by the chi-square test.

RESULTS

There was no difference in prevaccination seronegativity rates (titers < 8) between those with fourfold vaccine response and those without, comparing the student group and transplant group, for any of the virus strains tested.

The antibody response to the homologous influenza A strain is illustrated in Fig. 1. The diagonal lines in the figure represent, from top to bottom, fourfold, twofold, and no rise in antibody titer. Only 14 of 45 transplant patients (31%) demonstrated a fourfold or greater titer rise, compared with a significantly higher rate, 37 out of 66 (56%), in the control group (P = 0.01). Twenty-two transplant recipients showed no rise at all compared with 13 controls. When the response to a heterologous virus, A/Denver/1/72 (H3N2), was examined, there was an even poorer response rate in the transplant recipients (Fig. 2). A fourfold or greater titer rise was found in 40 of 66 controls (61%), but only in 8 of 45 transplant patients (18%) (P < 0.001). No rise was detected in 35 transplant patients and in 20 of the control group.

The antibody responses to vaccination with B/Massachusetts/1/71 against the homologous B strain is illustrated in Fig. 3. As with influenza A, a less favorable response by the transplant group against B antigen was noted. A fourfold or greater rise to the homologous virus occurred in 11 of 45 transplant recipients (24%)

Fig. 1. Homologous serum hemagglutination inhibition antibody response to A/Hong Kong/8/68 (H3N2).

Fig. 2. Heterologous serum hemagglutination inhibition antibody response to A/Denver/1/72 (H3N2).
versus 37 of 62 controls (60%) ($P < 0.001$). A similar pattern occurred with the heterologous B/452/73 virus. Thirty-three of 62 controls versus 9 of 45 transplant patients developed a fourfold or greater antibody rise ($P < 0.001$). Thirty (67%) and 33 (73%) of the 45 transplant recipients failed to show any rise in titer to B/Massachusetts/8/66 and B/452/73, respectively. In the control group, 13 (21%) and 23 (37%) of 62 vaccinees showed static titers to the homologous and heterologous strains, respectively.

Vaccination did not precipitate any transplant rejection phenomena, at least those measurable by blood urea nitrogen and serum creatinine determinations.

To examine what factors in the transplant group might have led to a less satisfactory antibody response, those who showed a fourfold or greater rise to the homologous A/Hong Kong/8/68 strain were compared with those who demonstrated a less than fourfold rise, with respect to age, length of time since transplantation, type and dose of immunosuppressive therapy, renal function, HL-A type, previous influenza vaccination, and whether their original renal disease resulted from an immune-mediated process, such as glomerulonephritis, or a non-immune-mediated process, such as polycystic disease or chronic pyelonephritis. These comparisons are outlined in Table 1. No significant difference could be recognized between those whose antibody titers did not rise fourfold and those whose did for any of these parameters, except for a greater rate of fourfold rises in patients with HL-A type W15 ($P < 0.05$).

**DISCUSSION**

In the winter of 1971–72, during an A/Hong Kong influenza outbreak in Scotland, Briggs et al. reported renal transplant rejection phenomena in three of five patients with influenzal illness (1). Despite immunosuppressive therapy, all five patients developed 16- to 128-fold complement-fixing antibody rises. In a subse-

**FIG. 3.** Homologous serum hemagglutination inhibition antibody response to influenza B.

**TABLE 1.** Comparative factors in transplant recipients with $\geq$4-fold and $<4$-fold homologous hemagglutination inhibition antibody rise to A/Hong Kong/8/68(H3N2)

<table>
<thead>
<tr>
<th>Antibody rise</th>
<th>No. of patients</th>
<th>Mean age (yr)</th>
<th>No. with previous influenza vaccine</th>
<th>% Initially seronegative</th>
<th>Mean time since transplant (mo)</th>
<th>Mean immunosuppressive dosage (mg/day)*</th>
<th>Renal function</th>
<th>HL-A W15</th>
<th>Mechanisms of renal disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq$4-fold</td>
<td>14</td>
<td>26.9</td>
<td>10</td>
<td>35.5</td>
<td>24.2 (12.5–30.0)</td>
<td>21.8 (12.5–125)</td>
<td>62.2 (12.5–100)</td>
<td>1.4</td>
<td>66.9 (25.9–111.0)</td>
</tr>
<tr>
<td>$&lt;4$-fold</td>
<td>31</td>
<td>27.0</td>
<td>2</td>
<td>42.5</td>
<td>33.8 (2.72)</td>
<td>21.6 (12.5–30.0)</td>
<td>61.5 (12.5–100)</td>
<td>1.7</td>
<td>65.8 (26.4–95.0)</td>
</tr>
</tbody>
</table>

* Four patients who received cyclophosphamide are not included. One of four developed $\geq$4-fold antibody rise.

* Numbers in parentheses represent range about the mean.

* Three of nine tested

* One of 21 tested

($P < 0.05$).
quent study of homologous antibody response to 400 IU of A/Hong Kong/1/68 (H3N2) vaccine in 25 renal transplant patients being maintained on 15 mg of prednisone and 100 to 200 mg of azathioprine per day, Carroll et al. found no difference in the degree of antibody response when compared with medical staff controls (2). In a more recent study, Pabico et al. demonstrated a retarded antibody response to A/Port Chalmers vaccine in renal transplant patients, with creatinine clearances less than 70 ml/min per m², versus those with a clearance greater than 70 ml/min per 1.73 m². However, no normal controls were compared with the transplant patients (9). These data are at variance with ours, which demonstrate a significantly retarded antibody response in transplant patients against both homologous and heterologous strains of influenza A and B, regardless of the level of renal function, when compared with healthy control subjects. Both impaired and normal antibody responses to influenza vaccination have been demonstrated in patients with renal impairment secondary to chronic glomerular disease, but vaccination per se did not appear to alter renal function (5, 8). Additional work has shown that there is a normal antibody response to parenteral influenza vaccine in patients with renal disease with normal creatinine clearance, but that azotemic patients respond poorly. Interestingly, hemodialysis patients respond to parenteral vaccine but not to live vaccine (R. F. Betts, R. G. Douglas, F. Roth, R. Pabico, and R. Freeman, Prog. Intersc. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 317, 1974). We could not distinguish a difference in the degree of antibody response between our patients with creatinine clearances of 70 ml/min or more (5 of 20 and 2 of 19 having a fourfold or greater rise to A/Hong Kong and A/Denver, respectively), and those with clearances less than 70 ml/min (8 of 23 and 5 of 24 having a fourfold or greater rise to A/Hong Kong and A/Denver, respectively). There was no difference in the dose of immunosuppressive drugs among those who developed a fourfold or greater response and those who did not. Azathioprine is known to suppress mainly the primary response, but not the secondary or anamnestic response, whereas cyclophosphamide suppresses both phases (4, 6). Since only four patients were receiving cyclophosphamide, a comparison of antibody response between azathioprine-prednisone and cyclophosphamide-prednisone regimens was not possible.

The association of immune response and HL-A type has been studied previously. Spencer et al. showed that type W16 was associated with a decreased antibody response to A/England/42/72 (H3N2) in normal subjects vaccinated with live "Alice" strain vaccine but not with inactivated vaccine (11). Our data on the increased antibody response in transplant patients with W15 as compared with those with other HL-A types are interesting but difficult to interpret since no HL-A typing was performed on the control group.

These data demonstrate that as a group, renal transplant patients have an impaired antibody response to inactivated influenza vaccine when compared with young healthy individuals. Double-dose vaccination or some type of adjuvant vaccination should be studied in this group to see if a more optimal antibody response rate can be achieved.

LITERATURE CITED