Antibody Response After Influenza Immunization in Renal Transplant Patients Receiving Cyclosporin A or Azathioprine

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Nineteen renal transplant recipients receiving cyclosporin A and prednisone, eight kidney recipients receiving azathioprine and prednisone, and 12 healthy volunteers were immunized with 0.5 ml of trivalent influenza vaccine containing A/Bangkok/1/79 (H_3N_2), A/Brazil/11/78 (H_1N_1), and B/Singapore/222/79. Nine patients (47%) in the cyclosporin A group and five (63%) in the azathioprine group showed fourfold rises in titer to at least one virus strain compared with 12 (100%) in the control group.

Cyclosporin A (CyA), a cyclic undecapeptide extracted from *Cylindrocarpon lucidum* and *Trichoderma polysporum*, was discovered in 1972 (1, 2) and has become an important antirejection agent that is now being used after graft surgery (7, 22). It selectively inhibits one or more T cell functions sparing T suppressor cells (12), and it inhibits production of interleukin 1 and 2 by macrophages and T lymphocytes (5). CyA suppresses such cell-mediated immune responses as graft rejection, graft versus host disease, delayed hypersensitivity skin reactions, and experimental allergic encephalomyelitis in rodents (1, 2).

Thymus-dependent but not T-independent humoral immune responses are inhibited in mice (1, 2). However, by using human lymphocytes, Paavonen and Häry (18) demonstrated that T-dependent and T-independent antibody responses were inhibited by CyA in vitro.

This study was undertaken to determine the effect of CyA on response to influenza vaccine in renal transplant recipients, in the hope that this immunosuppressant might permit normal or near-normal antibody responses. Influenza vaccination is strongly recommended for persons with chronic renal disease (15).

Three groups of individuals were studied. The first group (CyA group) consisted of 19 kidney transplant recipients who received CyA and prednisone as immunosuppressants. There were 8 males and 11 females who ranged from 18 to 56 years old (mean, 35.1 ± 2.6 years). Kidney transplants were performed 3 to 32 weeks (mean, 13.8 ± 2.0 weeks) before immunization. At the time of immunization, they received a mean dose of 11.6 mg of CyA per kg per day (range, 7.5 to 17.5 mg) and 16.8 mg of prednisone per day (range, 10 to 30 mg). The second

group (Aza group) consisted of eight kidney recipients who received a mean dose of 1.7 ± 0.25 mg of azathioprine (Aza) per kg per day (range, 0.38 to 2.68 mg) and 27.5 ± 3.3 mg of prednisone per day (range, 15 to 45 mg). The Aza group was composed of one male and seven females, ranging in age from 21 to 54 years (mean, 38.6 ± 4.5 years). Transplantations had been done 6 to 53 weeks (mean, 19.5 ± 5.5 weeks) before the study. The third group included 12 healthy volunteers who served as a control group. This group was composed of nine males and three females, whose mean age was 38.5 ± 3.3 years (range, 25 to 55 years).

Each subject received an intramuscular injection of 0.5 ml of zonal centrifuged ether-treated subvirion influenza vaccine. It contained 15 μg each of hemagglutinin of A/Bangkok/1/79 (H_3N_2), A/Brazil/11/78 (H_1N_1), and B/Singapore/222/79 strains (Parke-Davis, Morris Plains, N.J.). Blood samples were drawn immediately before and 4 to 5 weeks after immunization. Antibody titers were determined by the hemagglutination inhibition (HAI) test with Centers for Disease Control-derived A/Bangkok/1/79 (H_3N_2), A/Brazil/11/79 (H_1N_1), and B/Singapore/222/79 antigens kindly provided by the Allegheny County Health Department, Pittsburgh, Pa. All sera were tested against one specific antigen on the same day, according to the procedures described by the World Health Organization Collaborating Center for Influenza, Centers for Disease Control, Atlanta, Ga. (26).

The age distributions of both the CyA and Aza groups were similar to that of the control group. The Aza group consisted predominantly of females ($P < 0.05$ compared to control group), but sex proportions in the CyA and control groups were not significantly different. The proportions

TABLE 1. Frequency of response to antigens

Group	HAI titer[a]	Response rate[b]			
		A/Bangkok (H₃N₂)	A/Brazil (H₁N₁)	B/Singapore	At least one antigen
CyA	<1:10	3/6	1/2	3/4	
	≥1:10	5/13	5/17	2/15	
	Both	8/19[c] (42%)	6/19[d] (32%)	5/19[d] (26%)	9/19[c] (47%)
Aza	<1:10	1/1	1/1	0/1	
	≥1:10	2/7	4/7	2/7	
	Both	3/8[c] (38%)	5/8[e] (63%)	2/8[e] (25%)	5/8[e] (63%)
Control	<1:10	5/5	1/3	3/3	
	≥1:10	7/7	8/9	6/9	
	Both	12/12 (100%)	9/12 (75%)	9/12 (75%)	12/12 (100%)

[a] At time of vaccination.
[b] Number of patients with ≥ fourfold response of total number of patients in group.
[c] Significantly different from control ($P < 0.01$).
[d] Significantly different from control ($P < 0.05$).
[e] Not significantly different from control ($P > 0.05$) by Fisher's exact test, two-tailed.

of subjects with preimmunization titers of <1:10 for each of the three antigens were also not significantly different among the three groups.

Immunization was well tolerated by all subjects. Only local soreness was reported. Serum creatinine levels of patients remained stable during the study period; they were 1.83 ± 0.12 mg/dl before and 1.92 ± 0.13 mg/dl after immunization for the CyA group and 1.32 ± 0.16 mg/dl before and 1.30 ± 0.12 mg/dl after for the Aza group. Normal levels in our laboratories are <1.4 mg/dl for males and <1.2 mg/dl for females. Poor renal function may impair antibody response in kidney recipients (15, 19). Although most (22 of 27) patients in our series had mild elevations of serum creatinine level, no relationship to vaccine response could be observed. For instance, 8 of 15 patients (53%) with creatinine levels not more than 0.5 mg/dl above normal (mean of pre- and postimmunization determinations) responded to one or more antigens, and 6 of 12 (50%) with serum creatinine levels greater than 0.5 mg/dl above normal responded to at least one antigen.

The frequency of antibody responses is present in Table 1. Only 8 of 19 patients (42%) in the CyA group had significant antibody responses (fourfold or greater antibody titer rise) to A/ Bangkok virus, in comparison to 12 of 12 (100%) in the control group ($P < 0.01$). Frequencies of significant antibody responses to both A/Brazil and B/Singapore were also lower in the CyA group than in the control group ($P < 0.05$). Overall, only 9 of 19 CyA patients (47%) achieved fourfold or greater titer rises to at least one virus strain, compared to 12 of 12 controls ($P < 0.01$). Compared to the control group, the Aza also responded less well to the three antigens, but the difference in response was statistically significant only in the case of A/Bangkok ($P < 0.01$).

Preimmunization serological state may determine the responses to immunization (13, 25). However, the frequency of response in subjects with initial titers of <1:10 was not significantly different from the frequency in subjects with titers of ≥1:10. On the other hand, subjects in any group with titers >1:40 to an antigen had no significant antibody response to that antigen. This occurred in 14 instances. The results were therefore reanalyzed omitting these observations. The response rate and the magnitude of the response to B/Singapore in the CyA group and the magnitude of the response to B/Singapore in the Aza group were still significantly impaired. There were no subjects who had titers of >1:40 against A/Bangkok; hence, the significant reduction in the response to this antigen in the CyA and Aza groups was not affected.

The magnitude of HAI antibody rises and standard errors is shown in Fig. 1. The preimmunization titers were similar in the three groups, with an overall mean of 1:9.9. In addition to a lower frequency of significant antibody responses, the CyA group also had significantly lower titer rises to each of three antigens compared with the control group ($P < 0.01$). The Aza group also showed lower antibody responses to A/Bangkok and B/Singapore than the control group ($P < 0.05$), but the rise in titer against A/Brazil was not statistically different from that of the controls.

We conclude that patients receiving CyA or Aza responded less well to influenza immunization than controls. The observed difference was probably not, as shown above, due to age (18), prior immune status (13, 25), or poor renal function (15, 19), all factors which are known to
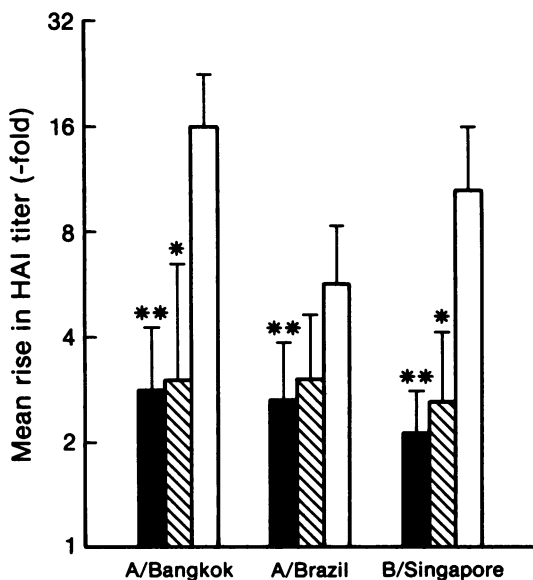
FIG. 1. HAI antibody rises in response to influenza vaccine. Bars represent the geometric mean changes (-fold) to each of the three antigens in the vaccine according to patient group. Solid bars, CyA; cross-hatched bars, Aza; open bars, control. Standard errors are indicated by lines. **, Significantly different from control group, $P < 0.01$; *, significantly different from control group, $P < 0.05$ by Student's t test.

modify immune response. However, all recipients received prednisone in addition to CyA or Aza.

Previous studies have shown that antibody responses after immunization with pneumococcal polysaccharide (17), typhoid vaccine (9), diphtheria toxoid (10), and influenza vaccine (16) are not inhibited by cortisone. Butler (6) showed that volunteers treated with 96 mg of methylprednisolone for 5 days had decreased immunoglobulin G levels, but there was no significant effect on antibody formation against a variety of antigens, including influenza vaccine. Brodman et al. (4) immunized 46 systemic lupus erythematosus patients with influenza vaccine, 23 of whom were receiving a mean dose of 20 mg of prednisolone per day. Their antibody responses were not significantly depressed. On the other hand, Herron et al. (11) noted depressed antibody response to influenza vaccine in patients receiving glucocorticords when compared with patients not receiving these drugs. Therefore, we cannot assume that prednisone played no role in the impaired antibody response of the CyA group.

Reports on the effect of Aza on the antibody response are not uniform. Briggs et al. (3) and Carroll et al. (8) reported that renal transplant recipients receiving Aza and prednisone were

able to mount a normal antibody response to influenza vaccine. However, other studies (15, 19, 21, 23) described impaired antibody responses among such patients. In some, the impaired responses were related to poor renal function (15, 19), but this relation could not be confirmed by others (3, 8, 21, 23). Despite fair renal functions, our Aza patients had a significantly lower response rate and smaller antibody rises than controls. In this respect, they were similar to the CyA group. If there is a difference in the responses of our Aza and CyA groups, the number of patients in the Aza group was too small to show it.

CyA is capable of suppressing antibody formation in animals (1, 2) and in in vitro human peripheral blood lymphocytes (18). Influenza hemagglutinin is probably a T-dependent antigen (23, 25). The suppression of the antibody response against this antigen shown in this study is consistent with the theory that CyA inhibits T-dependent antibody response (2).

The primary antibody response was more easily inhibited by CyA in animal studies than the secondary response. Borel et al. (2) showed that the primary antibody response to sheep erythrocytes in mice was depressed by 20 mg of CyA per kg per day, but optimal suppression of the secondary response required 300 mg/kg per day. In our study, it is difficult to ascertain whether the primary antibody response was suppressed by CyA, because the number of subjects with no preimmunization titer ($<1:10$) was small, and this group would be expected to include subjects with levels of immunity not detected by HAI. Since serotypes H_3N_2 and H_1N_1 have been circulating since 1968 and 1978, respectively, it is possible that all subjects in our study had been previously exposed to these antigens. However, antibody responses in those who were seropositive (titer of $\geq 1:10$) before immunization, certainly representing secondary antibody responses, were less in the CyA group than the control group (Table 1). Therefore, we suggest that secondary responses were depressed by CyA in our study.

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