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Humoral and Cellular Response in Humans After Immunization with Influenza Vaccine

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The peripheral blood lymphocyte response and hemagglutination inhibition antibody titers were measured in nine adults before and after immunization with a killed split influenza virus vaccine. Cord blood lymphocytes were tested with the influenza antigen to exclude a nonspecific mitogenic effect. All of the subjects demonstrated preexisting antibody titers and antigen recognition by lymphocytes prior to immunization. The in vitro lymphocyte response after vaccination parallels the humoral antibody response to influenza antigen.

The humoral antibody response to influenza vaccine has been well described, but there is little information on the cellular response to clinical influenza or in response to vaccination. This study examines the in vitro lymphocyte response and humoral antibody response to influenza vaccine before and after immunization.

MATERIALS AND METHODS

Subjects. Healthy medical and nursing student volunteers were screened for allergy to egg or egg products. They were bled, vaccinated, and finally rebled 10 days postvaccination. Cord blood samples were collected during uncomplicated deliveries.

Vaccine. A bivalent tri (n) butyl phosphate split inactivated influenza vaccine containing 400 chick cell-agglutination (CCA) units of influenza A/Aichi/68/H_3N_2, and 300 CCA units of influenza B/Mass/66 were given either intramuscularly or subcutaneously in the deltoid region of the arm.

Antibody determinations. Hemagglutination inhibition (HAI) antibody studies were done as previously described (4).

^{3}H-Thymidine studies. Approximately 30 ml of venous blood was drawn and mixed in a heparinized syringe. Plasma gel (Laboratory Roger Bellon, Neuilly, France) was added (1 ml for each 10 ml of blood) for sedimentation of red blood cells. The syringe was incubated 1 h at 37 C. Plasma and leukocytes were removed and cell count with differential was done. Individual 4-ml cultures containing 3×10^6 lymphocytes were set up in minimal essential media (Spinner, Grand Island Biological Co., Grand Island, N.Y.) with 12.5 to 20% autologous plasma, and penicillin (50 U/ml) and streptomycin (50 μg/ml; Microbiological Associates, Bethesda, Md.). Antigens or mitogens were added as follows: (i) phytohemagglutinin-P (PHA, Difco, Detroit, Mich.), 0.05 ml/culture; (ii) streptolysin-O (Difco), 0.5 ml/culture; (iii) monovalent tri (n) butyl phosphate split influenza vaccine (Wyeth Lab., Philadelphia, Pa.) at three concentrations: 1,920, 640, and 80 CCA of A/Aichi per ml. (Three different concentrations of influenza antigen for cultures were chosen, since it is well known that lymphocyte responsiveness may vary with the concentration of antigen used.)

Lymphocyte cultures were performed in triplicate and harvested at 72 h for PHA and 120 h for antigens. The cultures were pulsed with 2.0 μCi of tritiated ^{3}H-thymidine (New England Nuclear Corp.) 6 h (for PHA cultures) or 24 h (for cultures with antigen) before harvesting. The cells were processed and counted in a liquid scintillation counter. The counts were corrected for quenching, averaged, and expressed as counts per minute per culture or as the ratio of counts per minute with antigen to counts per minute in controls.

RESULTS

Cord bloods. Lymphocytes from five cord blood specimens showed no increase in response to influenza antigens in the 15 tests performed (Table 1). The ratio of cultures with antigen to cultures without antigen ranged from 1.0 to 1.9. In contrast, PHA responsiveness was demonstrated in three cultures, indicating the capability of the lymphocytes to proliferate in response to a nonspecific mitogen.

Antibody response in vaccinees. Seven of the nine students had a fourfold or greater rise in HAI antibody to A/Aichi (Table 2). One of the two without such a rise had a very high prevaccine antibody level (student 2), and the other (student 1) had a falling antibody titer. Unfor-

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tunately, there was insufficient serum to retest the latter student.

Antibody studies on the cord plasma specimens were not performed, since one would expect to find maternal transplacental antibody to influenza.

Streptolysin-O responses (not shown) revealed stimulation ratios of 14.0 to 200.5, with an average of 66.6. Two of seven with paired results (pre- and postvaccine) available showed some increases of postvaccine over prevaccine. The average value for prevaccine stimulation ratios was 82.0, and postvaccine was 46.7.

TABLE 1. *^{3}H-Thymidine incorporation in cord blood lymphocyte cultures stimulated with influenza A/Aichi antigen and PHA*

Cord blood no.	Control (counts/min × 10^3)	Ratio of tests/control			PHA
		1,920[a]	640[a]	80[a]	
1	1.1	1.8	1.5	1.3	5.7
2	1.5	1.2	1.1	1.2	19.3
3	1.5	1.4	1.4	1.3	
4	1.3	1.4	1.2	1.4	34.4
5	1.9	1.2	1.3	1.0	

[a] CCA units of A/Aichi antigen per milliliter.

Lymphocyte response in vaccinees. Lymphocytes from all of the subjects demonstrated a response to one or more concentrations of influenza antigen prior to vaccination by an increase in ^{3}H-thymidine incorporation (Table 2). Whereas the lymphocyte response was relatively low in subject 6 who had a titer of 1:4, the remaining subjects showed considerable variability in the magnitude of the response with different concentrations of antigen and with respect to the antibody titer. After vaccination, increases in thymidine incorporation compared with preimmunization values were observed in seven of the nine subjects. Subject 1 showed no response to influenza antigen after vaccination. Lymphocytes from subject 3 were responsive to all concentrations of antigen, but the counts were lower than those prior to immunization. In part, this is due to the higher counts in the postvaccination control cultures. Although the highest postvaccination counts tend to occur in subjects with greater increases in HAI antibody, the numbers are too small to draw any conclusions.

DISCUSSION

These studies indicate that adult human lymphocytes respond to influenza antigen in vitro. In

TABLE 2. *HAI antibody response and ^{3}H-thymidine incorporation in lymphocyte cultures before and after vaccination with influenza vaccine*

Student no.	Specimen	Antibody response			Lymphocyte response		
		Reciprocal of titer	Fold increase	Control (counts/min × 10^3)	Ratio of antigen/control		
					1,920[a]	640[a]	80[a]
1	Prevaccine	128	−4	0.4	0.9	68.8	104.2
	Postvaccine	32		1.0	0.1	2.5	0.2
2	Prevaccine	512	2	2.2	11.1		
	Postvaccine	1,024		2.8	21.7 (2)[b]	15.8	
3	Prevaccine	16	8	0.7	8.6	29.0	14.7
	Postvaccine	128		2.9	3.0	6.8	6.1
4	Prevaccine	64	8	1.6			23.9
	Postvaccine	512		1.4			46.5 (2)
5	Prevaccine	128	8	1.6	20.6	34.5	7.0
	Postvaccine	1,024		1.0	39.8 (2)	27.6	86.4 (12)
6	Prevaccine	4	32	0.8	4.9	2.8	2.9
	Postvaccine	128		0.7	19.4 (4)	34.5 (12)	22.7 (7)
7	Prevaccine	16	128	2.5	20.0		4.3
	Postvaccine	2,048		1.9	101.7 (5)	102.1	92.5 (21)
8	Prevaccine	16	128	5.0	5.8	6.7	7.7
	Postvaccine	2,048		3.2	12.0 (2)	20.0 (3)	27.2 (4)
9	Prevaccine	4	256	1.9	8.5	47.5	
	Postvaccine	1,024		1.3	125.0 (14)	206.8 (4)	84.2

[a] CCA units of A/Aichi antigen per milliliter.

[b] Numbers in parentheses refer to the ratio of postvaccine ratio to prevaccine ratio.

contrast, lymphocytes of the newborn do not proliferate in the presence of influenza antigen, indicating that these lymphocytes have not been previously sensitized to influenza. Similarly, cord blood lymphocytes did not respond to streptolysin-O, a specific antigen which requires prior sensitization for an in vitro response. The PHA responses show that these cord lymphocytes are capable of proliferating in the presence of a mitogen. The lack of cord blood responsiveness to influenza antigen contrasts with the adult prevaccine lymphocyte responsiveness. All of the subjects demonstrated both an in vitro lymphocyte response and humoral antibody, indicating prior sensitization with influenza or a cross-reacting antigen. After immunization with killed influenza vaccine, there is an increase in lymphocyte proliferation, in most cases paralleling a secondary antibody response. An in vitro lymphocyte response was also observed with streptolysin-O, but, in contrast to the responses to influenza antigen, there was no measurable increase in the 10-day specimens.

Cate (2) studied lymphocyte proliferation in adults 9 to 11 months after exposure to an influenza outbreak. He found a lymphocyte response only in persons with both a history of clinical influenza and previous vaccination. In his tests he used less than one CCA unit of influenza whole virus and obtained stimulation ratios, when positive, of 2 to 8. Thus, the low concentration of antigens may account for the lack of responsiveness in the groups receiving vaccination or with a history of clinical influenza.

Other studies were in animal models and measured other indicators of cell-mediated immunity. Feinstone (3) has shown an increase in migration inhibition factor values in mice after vaccination with whole influenza virus in Freund complete adjuvant. Waldman (5) has shown that guinea pigs given local spray or parenteral influenza vaccines have increased responses as measured by migration inhibition factor testing. Finally, Cate (1) demonstrated that mice receiving transfused lymphocytes from influenza A/PR 8-vaccinated mice have increased survival from challenge with the homologous virus.

The increased lymphoproliferative response to influenza antigens seen after vaccination suggests an increased immune response. Whereas the in vitro lymphocyte response is considered an antigen-recognition response, both B and T cells circulate in peripheral blood with the majority of lymphocytes as T cells. The magnitude of the in vitro lymphocyte response to influenza antigen implies some cell-mediated immune component in the response to influenza. The role of cellular versus humoral immunity has not been determined, but the accumulative evidence suggests that both may be important and deserve further study.

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LITERATURE CITED

1. Cate, T. R. 1971. Cell mediated immunity in influenza. Clin. Res. **19**:455.
2. Cate, T. R., and J. R. Kelly. 1970. Hong Kong influenza antigen sensitivity and decreased interferon response of peripheral lymphocytes. Antimicrob. Ag. Chemother. 1969, p. 156–160.
3. Feinstone, S. M., E. H. Beachy, and M. W. Rytel. 1969. Induction of delayed hypersensitivity to influenza and mumps viruses in mice. J. Immunol. **103**:844–849.
4. Ruben, F. L., and G. G. Jackson. 1972. A new subunit influenza vaccine acceptability compared with standard vaccines and antigenicity in increasing dosage. J. Infect. Dis. **125**:656–664.
5. Waldman, R. H., C. S. Spencer, and J. E. Johnson. 1972. Respiratory and systemic cellular and humoral immune responses to influenza virus vaccine administered parenterally or by nose drops. Cell Immunol. **3**:294–300.