INFECTION AND IMMUNITY, July 1979, p. 103-109
0019-9567/79/07-0103/07$02.00/0

Vol. 25, No. 1

Cell-Mediated Immune Responses to the Hemagglutinin and Neuraminidase Antigens of Influenza A Virus After Immunization in Humans

TINA C. CHOW,[1,2] KARL R. BEUTNER,† AND PEARAY L. OGRA[1,2]*

Division of Infectious Disease and Virology,[1] Children's Hospital, and Departments of Pediatrics and Microbiology,[2] State University of New York at Buffalo, Buffalo, New York 14222

Received for publication 26 March 1979

Humoral and cell-mediated immunity (CMI) were evaluated in groups of school children after immunization with inactivated influenza virus vaccines. A conventional biphasic strain (H_3ChN_2Ch) of Port Chalmers influenza virus (X-41), a recombinant influenza virus specific for the neuraminidase antigen (Heq_1N_2Ch) of Port Chalmers influenza A virus (X-42), and a placebo were employed for immunization. The techniques of hemagglutination inhibition and neuraminidase inhibition were used to determine serum antibody titers. The CMI responses were evaluated by the in vitro lymphocyte transformation assay employing $HavN_2Ch$, Heq_1Neq_1, H_3ChNeq_1, and H_3ChN_2Ch influenza A virus strains as stimulants. Specific HAI antibody and CMI responses to H_3Ch were observed in X-41 but not in X-42 vaccinees. Specific anti-neuraminidase antibodies and CMI responses to N_2Ch were manifested by both X-41 and X-42 vaccinees. Immunization with the placebo resulted in no influenza-specific immune responses. The CMI response was first detectable 10 days after immunization and then declined. These observations demonstrate the induction of CMI responses to the HA and NA influenza surface antigens after immunization. These responses may be important in antiviral immunity and the recovery from influenza infection.

The antibody response to influenza virus after natural infection or immunization has been studied extensively (4, 11, 19, 23-25, 32). It has been proposed that antibodies to the hemagglutinin (HA) antigen of influenza protect individuals against influenza infection, while antibodies to the neuraminidase (NA) antigen may protect against illness but not infection (12, 13, 18, 24).

The possibility that cell-mediated immunity (CMI) may play a role in the pathogenesis or protection against influenza has also been suggested. Several lines of evidence support this concept. After the disappearance of antibody, mice were still protected against a lethal challenge of influenza virus (15). Susceptibility to influenza infection in the presence of serum antibody and resistance to influenza infection in the absence of detectable serum antibody have also been observed in other studies (26). These results suggest the existence of a host defense mechanism other than antibody which may be operative in influenza immunity or pathogenesis.

Recently influenza-specific CMI activity has been observed after immunization in humans (22). These studies employed the immunizing virus as the test antigen. However, little information is available regarding HA- and NA-specific influenza CMI response in humans. The purpose of the present study was to evaluate the specificity and temporal kinetics of the CMI response to the HA and NA surface antigens of influenza A virus after immunization with inactivated influenza A virus vaccines.

† Present address: Department of Pediatrics, University of California, Los Angeles, School of Medicine, Los Angeles, CA 90024.

MATERIALS AND METHODS

Study population. The study population consisted of 12 healthy children from the Buffalo Public School system. These children were part of a larger group participating in an influenza virus vaccine trial. Informed written consent for immunization and subsequent testing was obtained from the parents or guardians of these children. This study was reviewed and approved by the Buffalo Children's Hospital Human Experimentation Committee.

Specimens of heparinized blood and serum were collected from subjects before immunization and at 10, 30, and 80 days after vaccination. Heparinized blood specimens were processed as described below. All serum samples were stored at −20°C before testing for influenza-specific antibody activity.

In addition, seven specimens of heparinized cord blood and heparinized blood samples from seven healthy adults seropositive for H_2ChN_2Ch antibody were also tested as described below.

Vaccines. Three vaccines were employed for these

investigations: X-41, X-42, and a placebo. X-41 is a conventional inactivated Port Chalmers (H_3ChN_2Ch) influenza vaccine. X-42 is the recombinant virus vaccine produced by the genetic hybridization of equine and Port Chalmers strains of influenza virus. The progeny strain employed for immunization contained equine HA (Heq_1) and Port Chalmers NA (N_2Ch) antigens. Vaccine diluent was employed as the placebo control. The hemagglutination titers of the X-41 and X-42 vaccines were 3,072 and 1,024, respectively, as determined by the precision fractional dilution technique (30). The X-41 vaccine contained 2.3-fold more NA activity than the X-42 vaccine as determined by the NA assay. All vaccine preparations were produced by Merrell-National Laboratories, Cincinnati, Ohio. Subjects were inoculated with a single dose of 0.5 ml of the appropriate vaccine.

Virus strains. The following influenza virus strains were employed for this study: A_2/Port Chalmers/73 (H_3ChN_2Ch); A_2/Port Chalmers/73-A/Equine/56 (H_3ChNeq_1); A/Equine/56-A_2/Port Chalmers/73 (Heq_1N_2Ch); A/Avian-A/Port Chalmers/73 ($HavN_2Ch$); and A/Equine/56 (Heq_1Neq_1). All viruses were grown in embryonated eggs from seed stocks initially furnished by E. D. Kilbourne and J. Schulman (Mt. Sinai School of Medicine, New York, N.Y.) and R. G. Webster (St. Jude Children's Research Hospital, Memphis, Tenn.). Virus pools of each strain were prepared by allantoic inoculation of embryonated eggs. After incubation at 35°C for 48 h, the eggs were chilled at −20°C for 1 h and the allantoic fluid was harvested and pooled. The allantoic fluid from infected eggs was then clarified by centrifugation at 500 × *g* for 30 min at 4°C and stored at −85°C.

Serological procedures. Purified and crude influenza virus preparations were quantitated by assaying for influenza virus HA and NA activity. The assay for the influenza virus HA was performed by using a standard microtiter procedure (7). The NA activity of influenza virus preparations was determined as previously described (1, 3, 4, 36).

Antibody activity to influenza HA was detected by a standard microtiter hemagglutination inhibition (HAI) assay employing receptor-destroying enzyme pretreatment, 4 HA units (HAU) of influenza virus, and chicken erythrocytes as indicator cells. Samples collected from the vaccines were tested for specific H_3Ch and Heq_1 antibody activity by using the recombinant H_3ChNeq_1 and Heq_1Neq_1 strains, respectively, as the test antigens. Anti-NA antibody (ANAB) titers were determined by employing an enzyme inhibition test as previously described (35, 36). The recombinant $HavN_2Ch$ influenza virus was employed in the ANAB assay.

Purification of influenza virus. The recombinant strains of influenza virus H_3ChN_2Ch, H_3ChNeq_1, $HavN_2Ch$, and Heq_1Neq_1 were purified and employed as stimulants in the in vitro lymphocyte transformation assay. For purification, 30 ml of allantoic fluid was layered onto a 0.8-ml cushion of 25% sucrose (wt/vol) and centrifuged in a Beckman model L-50 ultracentrifuge by using an SW-25 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) at 47,000 × *g* for 1 h. The resulting pellet was diluted 1:4 with phosphate-buffered saline (PBS), pH 7.4, and 20 ml of this preparation was layered onto a 30 to 60% sucrose (wt/vol) gradient in PBS and centrifuged in an SW-25 rotor at 47,000 × *g* for 1 h. The visible band of virus was collected, dialyzed against three changes of PBS at 4°C for 48 h, and concentrated by centrifugation at 47,000 × *g* for 1 h. The pellet was resuspended in PBS and layered onto a sucrose gradient, and the process was repeated as described above.

The pooled, dialyzed influenza virus preparations were filtered through a 0.45-μm membrane filter (Millipore Co., Bedford, Mass.) inactivated by exposure to ultraviolet light for 30 min, aliquoted, and stored at −85°C. The HA titers of the purified virus ranged from 8×10^4 to 2×10^5 HAU per ml. As a control antigen, uninfected allantoic fluid was purified by the procedure described above and diluted 1:10 in RPMI-1640.

LTF assay. The lymphocyte transformation (LTF) assay employed in this study was a micro-whole blood culture system as described by Pauly and Han (20, 21). After the determination of a total whole blood cell count and a differential count, samples of whole blood were diluted in serum-free RPMI-1640 medium (GIBCO, Grand Island, N. Y.) supplemented with glutamine and containing kanamycin (60 μg/ml) to a final lymphocyte concentration of 2×10^5/ml. A 0.2-ml volume of the cell suspension was added to U-bottom wells of microtiter plates (Cooke Laboratory Products, Alexandria, Va.). Before the addition of cells, 0.02 ml of uninfected allantoic fluid or an appropriate dilution of influenza antigen was added to triplicate sets of wells. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. On day 5 of culture, 0.2 μCi of [*methyl*-^{3}H]thymidine (specific activity, 1.9 μCi/mM; Schwarz/Mann, Orangeburg, N.Y.) in 0.02 ml of RPMI-1640 was added to each culture.

After an additional 18 h of incubation, the cells were harvested with a Multiple Automated Sample Harvester (model II, Microbiological Associates, Bethesda, Md.). The cells were washed with ca. 4 ml of 3% acetic acid and finally deposited onto a glass fiber strip (Reeves Angele, Clifton, N. J.). A drop of 30% hydrogen peroxide was appliedto each disk with a Pasteur pipette and allowed to dry at room temperature for 30 min. The samples were then processed for liquid scintillation counting. First, 0.4 ml of the tissue solubilizer (Protosol, New England Nuclear Corp., Boston, Mass.) was added to each vial. The vials were placed in a drying oven at 85°C for 20 min. After cooling, 9.6 ml of diluted toluene-based Liquafluor (New England Nuclear) was added. Before counting in a liquid scintillation counter (Beckman, model LS 250), the vials were allowed to dark adapt for 24–48 hours. The results of the LTF assay were expressed as stimulation indexes (SI), which were defined as the mean counts per minute of the stimulated lymphocytes divided by the mean counts per minute of unstimulated lymphocytes. An SI of 3 or greater was considered as evidence of in vitro lymphocyte stimulation.

RESULTS

Humoral antibody response. Serum antibody responses to H_3Ch and N_2Ch are presented

in Table 1. In each group, subjects were initially seronegative or manifested low titers of H_3Ch or N_2Ch antibody activity before immunization. Immunization with X-41 vaccine resulted in seroconversion for H_3Ch and N_2Ch antibody 10 days after immunization. Immunization with X-42 vaccine induced no increase in H_3Ch antibody activity, but all of the X-42 vaccinees manifested increases in N_2Ch antibody titers. No antibody response was observed in the placebo group.

The geometric mean titers of H_3Ch HAI antibody and N_2Ch ANAB were 16 and 7 before immunization and 98 and 41 at 10 days after immunization, respectively, in the X-41 group. The mean H_3Ch HAI titer before immunization was 10, and no increase was observed in X-42 vaccinees after immunization. However, the mean N_2Ch-specific ANAB titers increased from 10 to 343 after immunization with X-42 vaccine. The individual and mean antibody titers for N_2Ch-specific ANAB after immunization with X-42 vaccine were 4- to 10-fold higher than N_2Ch antibody titers observed after immunization with X-41 vaccine. Although some slight variation was observed, the individual and geometric mean HAI antibody and ANAB titers were remarkably similar on days 10, 30, and 80.

Lymphocyte responses to influenza antigens. The responses of lymphocytes from seven cord blood specimens tested with H_3ChN_2Ch influenza virus at different concentrations are shown in Fig. 1. No significant responses (SI > 3) were observed in any of the specimens tested.

Lymphocytes from healthy adult volunteers naturally infected with influenza virus H_3ChN_2Ch were used to determine the optimal antigen concentration for in vitro stimulation. The maximum responses were observed at concentrations ranging from 80 to 2,560 HAU per ml (Fig. 2). The concentration which elicited the maximum response varied from individual to individual within this range. In view of the variation of SI between the different antigen concentrations, influenza antigen preparations diluted in RPMI-1640 medium at 80, 640, and 2,560 HAU per ml were chosen as the concentrations to be employed for the LTF assay. The highest SI observed for a given individual regardless of the antigen concentration was considered as the individual's optimal response.

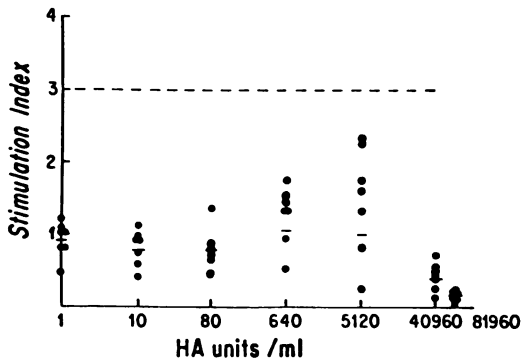

FIG. 1. *Individual (●) and mean (–) SI of cord blood lymphocyte cultures stimulated with H_3ChN_2Ch at different antigen concentrations. (---) Indicates an SI of 3.*

TABLE 1. *Serum HAI antibody and ANAB responses to H_3Ch and N_2Ch in subjects studied for CMI to influenza virus*

Group (vaccine)	Subject	H_3Ch HAI antibody titer at day:				N_2Ch ANAB titer at day:			
		Pre	10	30	80	Pre	10	30	80
X-41 (H_3ChN_2Ch)	L-3	<8	256	64	256	2	10	27	91
	L-7	16	128	128	NT[a]	10	147	128	NT
	L-11	16	64	64	64	16	64	111	208
	L-12	8	32	64	64	7	90	28	21
	L-16	64	128	128	64	8	14	22	24
	GMT[b]	16	98	85	91	7	41	47	56
X-42 ($Heq1N_2Ch$)	L-5	<8	<8	<8	8	23	548	512	588
	L-9	16	16	16	16	11	128	147	223
	L-10	<8	<8	8	8	2	415	362	445
	L-13	<8	<8	<8	8	16	477	445	362
	GMT	10	6	10	10	10	343	331	381
Placebo (diluent)	L-4	16	16	16	16	23	9	15	21
	L-6	<8	<8	<8	8	2	2	2	2
	L-15	16	16	16	16	4	10	2	10
	GMT	13	13	13	13	6	6	3	11

[a] NT, Not tested.
[b] GMT, Geometric mean titer.

Proliferative responses of peripheral blood lymphocytes obtained from the three groups of vaccinated subjects after in vitro stimulation with H_3ChN_2Ch (Port Chalmers) antigen are presented in Table 2. Ten days after vaccination, significant lymphoproliferative activity was observed in four of five X-41 (H_3ChN_2Ch)- and in three of four X-42 (Heq_1N_2Ch)-vaccinated subjects. Subjects L-12 (X-41) and L-13 (X-42) failed to respond to the H_3ChN_2Ch antigen despite their seroconversion for specific antibody (Table 1). The CMI response to H_3ChN_2Ch antigen gradually decreased after manifesting peak levels 10 days after immunization. No proliferative activity to H_3ChN_2Ch was observed 80 days after immunization in the X-42 group, whereas at 80 days several X-42 vaccinees manifested H_3ChN_2Ch-specific LTF activity.

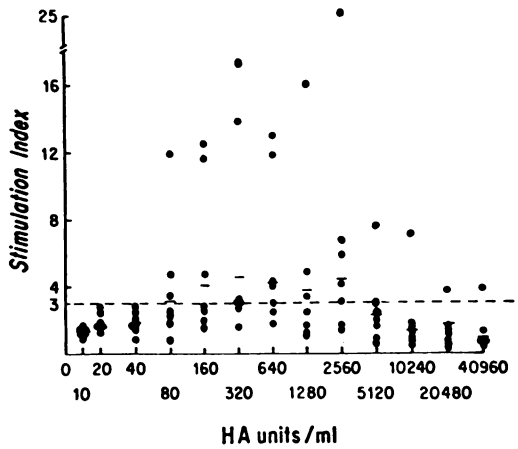

FIG. 2. *Individual (●) and mean (–) SI of lymphocyte cultures from seropositive naturally infected adults stimulated with H_3ChN_2Ch influenza virus at different concentrations. (---) Indicates an SI of 3.*

In vitro proliferative response to H_3Ch antigen was evaluated by testing with the recombinant H_3ChNeq_1 influenza virus. Ten days after immunization, four of five X-41 vaccinees manifested a significant LTF response to H_3ChNeq_1 (Table 2). The CMI response to H_3Ch antigen gradually decreased after the peak activity at day 10. Lymphoproliferative activity to the HA of Port Chalmers influenza virus (H_3Ch) was not significantly increased ($P > 0.05$) in the X-42 group after immunization with Heq_1N_2Ch vaccine. No response was observed in placebo subjects after in vitro stimulation with H_3ChNeq_1.

$HavN_2Ch$ served as a test antigen to evaluate N_2Ch specific lymphoproliferative responses (Table 3). Ten days after immunization, four of five X-41 recipients and all X-42 vaccinated subjects manifested significant LTF responses to $HavN_2Ch$. Although both groups were immunized with recombinant virus preparations con-

TABLE 2. *Individual and mean SI of lymphocyte cultures in response to influenza viral antigens H_3ChN_2Ch and $H_3ChNeq1$*[a]

Group (vaccine)	Subject	SI at days indicated: Test antigen H_3ChN_2Ch Pre	10	30	80	$H_3ChNeq1$ Pre	10	30	80
X-41	L-3	2.5	**9.3**	2.5	**5.7**	2.3	**8.9**	2.7	2.2
(H_3ChN_2Ch)	L-7	1.6	**11.2**	**4.8**	NT[b]	1.5	**10.8**	1.8	NT
	L-11	1.3	**6.2**	**6.9**	1.2	**3.5**	**5.9**	**3.1**	0.7
	L-12	0.9	1.5	2.0	**3.5**	0.8	1.7	1.8	2.3
	L-16	**4.4**	**20.6**	**10.5**	**5.5**	**5.1**	**17.9**	2.2	4.7
	GMI[c]	1.8	**7.3**	**4.6**	**3.4**		**7.0**	2.3	2.0
X-42	L-5	**3.1**	**9.7**	2.2	2.1	1.4	1.8	2.6	2.1
($Heq1N_2Ch$)	L-9	2.2	**6.9**	1.4	1.5	2.1	2.6	2.0	2.6
	L-10	**4.1**	**8.4**	**7.3**	2.8	2.2	**4.9**	**4.4**	2.8
	L-13	1.6	2.5	0.9	0.8	1.5	2.8	2.4	1.8
	GMI	2.6	**6.1**	2.1	1.6	1.8	2.8	2.7	2.3
Placebo	L-4	**3.5**	**3.6**	2.1	2.4	**3.6**	**3.5**	2.4	**3.0**
(diluent)	L-6	1.2	1.1	0.9	2.9	1.1	1.9	0.7	2.8
	L-15	1.4	0.6	1.0	1.3	1.7	1.0	1.4	2.6
	GMI	1.8	1.4	1.2	2.1	1.9	1.9	1.3	2.8

[a] Significant responses are in boldface.
[b] NT, Not tested.
[c] GMI, Geometric mean index.

TABLE 3. *Individual and mean SI of lymphocyte cultures in response to influenza viral antigens $HavN_2Ch$ and $Heq1Neq1$*[a]

Group (vaccine)	Subject	SI at days indicated							
		Test antigen							
		$HavN_2Ch$				$Heq1Neq1$			
		Pre	10	30	80	Pre	10	30	80
X-41 (H_3ChN_2Ch)	L-3	**3.4**	**7.5**	2.4	3.0	1.6	2.8	2.0	2.8
	L-7	1.6	**3.6**	1.8	NT[b]	1.3	1.7	**3.0**	NT
	L-11	1.8	**3.3**	**4.4**	1.3	1.6	2.5	1.1	1.8
	L-12	0.9	1.7	1.4	1.5	0.8	1.1	1.9	0.7
	L-16	**3.5**	**6.4**	**6.8**	**5.3**	2.8	1.9	1.6	1.9
	GMI[c]	2.0	**4.0**	**3.1**	2.4	1.5	1.9	1.6	1.6
X-41 ($Heq1N_2Ch$)	L-5	2.2	**12.6**	**3.1**	1.8	2.8	**4.4**	1.9	1.4
	L-9	1.5	**8.4**	2.1	**3.4**	1.5	**3.6**	**3.2**	2.4
	L-10	**4.9**	**41.0**	**17.6**	**5.8**	**4.3**	**7.0**	**5.2**	2.2
	L-13	1.7	**3.2**	1.6	2.0	1.8	2.6	2.1	2.2
	GMI	2.3	**10.8**	**3.7**	2.9	2.4	**4.1**	2.9	2.0
Placebo (diluent)	L-4	**3.9**	**4.6**	**4.1**	**3.0**	2.0	**3.6**	**3.0**	2.1
	L-6	0.7	1.7	0.7	1.9	0.7	1.2	0.9	1.2
	L-15	2.3	0.8	1.0	2.0	1.0	1.4	2.4	1.0
	GMI	1.8	1.9	1.4	2.3	1.1	1.8	1.9	1.4

[a] Significant responses are in boldface.
[b] NT, Not tested.
[c] GMI, Geometric mean index.

taining N_2Ch antigen, the mean and individual lymphoproliferative response to $HavN_2Ch$ antigen observed in X-42 (Heq_1N_2Ch) subjects were notably higher than the responses observed in X-41 (H_3ChN_2Ch) vaccinees. The temporal pattern of the LTF response to N_2Ch antigen was characterized by the appearance of peak activity 10 days after immunization. Subsequently, the LTF reactivity declined and only minimal activity was detectable 80 days after immunization. No response to $HavN_2Ch$ was manifested by the placebo group.

A detectable in vitro proliferative response to Heq_1 with Heq_1Neq_1 as the test antigen was observed only in X-42 subjects immunized with Heq_1N_2Ch vaccine (Table 3). Little or no proliferative activity to Heq_1Neq_1 was observed before immunization in the X-42 group, while three of four vaccinees manifested significant responses 10 days after immunization with Heq_1N_2Ch vaccine. This response was transient, being undetectable 30 to 80 days after immunization. No Heq_1Neq_1-specific in vitro lymphoproliferative response was observed in X-41 or placebo groups.

DISCUSSION

The serological responses of the vaccine groups indicate that all subjects were successfully immunized. The X-41 (H_3ChN_2Ch) vaccinees manifested seroconversion for H_3Ch- and N_2Ch-specific antibody. The recipients of the X-42 vaccine (Heq_1N_2Ch) manifested mean N_2Ch-specific antibody responses eightfold greater than those observed in X-41 (H_3ChN_2Ch) vaccinees. This would indicate that both vaccinees are immunogenic and that the X-42 vaccine is superior to the X-41 vaccine in eliciting antibodies to N_2Ch. This agrees with previous studies of the serological responses to these recombinant influenza vaccinees (19).

Lymphocyte transformation correlates directly with prior exposure to antigen and is dependent upon immunological memory of the cell population (31). This technique was employed to evaluate the CMI responses in the present study. Although the induction of lymphoproliferative activity by mitogens has been shown to involve both T- and B-cells (6), the initial triggering of proliferative responses to bacterial (purified protein derivative) and viral antigens (mumps and rubella) has been demonstrated to be primarily a T-cell response (6, 16).

The antigen concentrations employed for in vitro stimulation in the present study were determined in healthy seropositive adults. From these antigen dose response studies, optimal antigen concentrations were observed in the range of 80 to 2,560 HAU per ml. Variability in the antigen concentration at which in vitro lympho-

proliferation is observed has been reported in other studies on CMI responses to viral infections (3, 10).

The specificity of the LTF assay was confirmed by testing cord blood lymphocytes with the purified influenza virus and the use of heterologous antigens. Cord blood lymphocytes did not manifest significant lymphoproliferative responses to the influenza antigens. This indicates that these antigen preparations did not have nonspecific mitogenic activity. Influenza viruses Heq_1Neq_1 and H_3ChNeq_1 served as heterotypic antigens for the H_3ChN_2Ch (X-41) and Heq_1N_2Ch (X-42) vaccinees, respectively. Only two borderline proliferative responses to the heterotypic antigens were observed in the vaccine groups. These responses may have been elicited by the internal antigens shared by the stimulating and the immunizing virus. Alternatively, they could be manifestations of past influenza virus infections.

The HA- and NA-specific antibody responses after immunization with inactivated influenza vaccines has been extensively studied (4, 11, 19, 23–25, 32). However, little is known about the HA- and NA-specific CMI responses after such immunization. The present study has demonstrated that, after immunization with H_3ChN_2Ch influenza vaccine, specific H_3Ch and N_2Ch CMI responses are regularly detectable. N_2Ch-specific CMI responses are also detectable after vaccination with the NA-specific vaccine (Heq_1N_2Ch). CMI responses to the H_3Ch antigen were not augmented by immunization with Heq_1N_2Ch. Regardless of the influenza strain employed for immunization, the duration of detectable CMI responses appeared to be short-lived. The temporal pattern of the CMI responses to H_3Ch and N_2Ch antigens was characterized by the appearance of peak LTF activity 10 days after immunization. Subsequently, the lymphoproliferative responses declined and activity was undetectable after 30 to 80 days. Short-lived appearance of in vitro correlates of viral CMI has also been observed by other investigators in responses to influenza virus (5, 8, 34), rubella virus (17, 27), and Sendai virus (2).

The in vitro LTF assay is considered an antigen-recognition response (31). The increased lymphoproliferative response to influenza surface antigen seen after vaccination implies an increase in antigen recognition by peripheral blood lymphocytes in vitro. Both T- and B-cells circulate in peripheral blood, with the majority of lymphocytes as T-cells (9, 14). The initial triggering of the proliferative response to viral antigens has been demonstrated to be primarily a T-cell response (6, 16). A requirement for a T-cell helper activity to elicit anti-HA antibody production after influenza infection has been demonstrated (33). HAI antibody has been shown to protect humans against influenza virus infection, while protection against illness but not infection may be provided by ANAB (12, 13, 18, 24). Thus, sensitized T-lymphocytes, through their regulatory and helper activity on the humoral response, could have an essential role in the host defense mechanism against influenza infection.

The role of T-lymphocytes in the host defense against influenza may involve factors other than their participation in antibody formation. Local and systemic influenza-specific CMI has been considered an important factor in the pathogenesis of influenza virus infection and host defense mechanism in the recovery from influenza infections. Sullivan et al. (28) demonstrated that T-lymphocytes are responsible for limiting the dissemination of influenza virus in the lung. The T-lymphocytes have also been shown to be involved in the inflammatory response observed in lung tissue after influenza infection (38).

This study has demonstrated specific CMI responses to the HA and NA antigens of influenza virus after systemic influenza immunization, although the role of T-cells and specific CMI responses in the pathogenesis or recovery from influenza virus infection remains to be clearly defined.

ACKNOWLEDGMENT

This work was supported by Public Health Service contract AI-42511 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Aminoff, E.** 1961. Methods for quantitative estimation of N-acetyl-neuraminic acid and their application to hydrolysates of sialomucoids. Biochem. J. **81**:384–392.
2. **Anderson, M. J., D. R. Bainbridge, J. R. Pattison, and R. B. Hearth.** 1977. Cell-mediated immunity to Sendai virus infection in mice. Infect. Immun. **15**:239–244.
3. **Beutner, K. R., M. L. Tiku, and P. L. Ogra.** 1978. Hepatitis B surface antigen-specific cell-mediated immune responses in human chronic hepatitis B surface antigen carriers. Infect. Immun. **21**:480–488.
4. **Brown, G. C., and T. P. O'Leary.** 1973. Fluorescent antibodies to influenza virus in various immunoglobulin fractions of serum after natural infection or vaccination. J. Immunol. **110**:889–896.
5. **Cambridge, G., J. S. Mackenzie, and D. Keast.** 1976. Cell-mediated immune response to influenza virus infection in mice. Infect. Immun. **13**:36–43.
6. **Chess, L., R. P. MacDermott, and S. F. Schlossman.** 1974. Immunologic functions of isolated human lymphocyte subpopulations. II. Antigen triggering of T and B cells in-vitro. J. Immunol. **113**:1113–1122.
7. **Dowdle, W. R., W. M. Marine, M. Coleman, and V. Knez.** 1972. Hemagglutinin relationships of Hong Kong (H_3) and Asian (H_2) influenza strains delineated by antigen-specific recombinants. J. Gen. Virol. **16**:127–134.
8. **Gadol, N., J. E. Johnson III, and R. H. Waldman.**

1974. Respiratory tract cell-mediated immunity: comparison of primary and secondary response. Infect. Immun. 9:858–862.

9. **Jondall, M., G. Holm, and H. Wigzell.** 1972. Surface marker on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. J. Exp. Med. **136**:207–215.
10. **Jordan, G. W., and T. C. Merigan.** 1974. Cell-mediated immunity to varicella-zoster virus: in vitro lymphocyte response. J. Infect. Dis. **130**:495–501.
11. **Kilbourne, E. D., W. T. Butler, and R. D. Rossen.** 1973. Specific immunity in influenza: summary of influenza workshop. III. J. Infect. Dis. **127**:220–236.
12. **Kilbourne, E. D., W. G. Laver, J. L. Schulman, and R. G. Webster.** 1968. Antiviral activity of antiserum specific for an influenza virus neuraminidase. J. Virol. **2**:281–188.
13. **Kilbourne, E. D., J. L. Schulman, R. B. Couch, and J. A. Kasel.** 1972. Monoantigenic immunization and the induction of partial immunity. A new approach to influenza immunization, p. 118–119. *In* J. L. Melnick (ed.), International virology 2. Proceedings of the 2nd International Congress for Virology, S. Karger, Basel.
14. **Lay, W. H., N. F. Mendes, C. Bianco, and V. Nussenzweign.** 1971. Binding of sheep red blood cells to a large population of human lymphocytes. Nature (London) **230**:531–532.
15. **Loosli, C. G., D. Hamre, and B. S. Berlin.** 1953. Airborne influenza virus: an infection in immunized animals. Trans. Assoc. Am. Physicians **66**:222–230.
16. **Morag, A., J. M. Bernstein, B. Morag, K. R. Beutner, and P. L. Ogra.** 1975. In-vitro correlates of cell-mediated immunity in human tonsils after natural or induced rubella virus infection. J. Infect. Dis. **131**:409–416.
17. **Morag, A., K. R. Beutner, B. Morag, and P. L. Ogra,** 1974. Development and characteristics of in vitro correlates of cell-mediated immunity to rubella virus in the systemic and mucosal sites in guinea pigs. J. Immunol. **113**:1703–1709.
18. **Murphy, B. R., J. A. Kasel, and R. M. Chanock.** 1972. Association of serum anti-neuraminidase antibody with resistance to influenza in man. N. Engl. J. Med. **268**: 1329–1332.
19. **Ogra, P. L., K. R. Beutner, E. Rubi, J. Strussenberg, S. DeMello, and C. Rizzone.** 1977. Clinical and immunologic evaluation of neuraminidase specific influenza A vaccine in man. J. Infect. Dis. **135**:499–506.
20. **Pauly, J. L.** 1976. A simple screening assay for substances effecting lymphocyte reactivity. J. Immunol. Methods **12**:141–147.
21. **Pauly, J. L., and T. Han.** 1976. Whole blood microculture assay of human lymphocyte function. J. Lab. Clin. Med. **88**:864–872.
22. **Ruben, F. L., G. G. Jackson, and S. P. Gotoff.** 1973. Humoral and cellular response in humans after immunization with influenza vaccine. Infect. Immun. **7**:594–596.
23. **Schild, G. C., and R. W. Newman.** 1969. Antibody against influenza A_2 virus neuraminidase in human sera. J. Hyg. **67**:353–365.
24. **Schulman, J. L., M. Khakpour, and E. D. Kilbourne.** 1968. Protective effects of specific immunity to viral neuraminidase on influenza virus infection of mice. J. Virol. **2**:778–786.
25. **Shuartsman, Y. S., and M. P. Zykov.** 1976. Secretory anti-influenza immunity. Adv. Immunol. **22**:291–330.
26. **Small, P. A., Jr., R. H. Waldman, J. C. Bruno, and G. E. Gifford.** 1976. Influenza infection in ferrets: role of serum antibody in protection and recovery. Infect. Immun. **13**:417–424.
27. **Steele, R. W., S. R. Hense, and M. M. Vincent.** 1974. Development of specific cellular and humoral immune responses in children immunized with live rubella virus vaccine. J. Infect. Dis. **130**:449–453.
28. **Sullivan, J. L., R. E. Mayner, D. W. Barry, and F. A. Ennis,** 1976. Influenza virus infection in nude mice. J. Infect. Dis. **133**:91–94.
29. **Suzuki, E., J. Ohya, and N. Ishida.** 1974. Effect of antilymphocyte serum on influenza virus infection. Proc. Soc. Exp. Biol. Med. **146**:78–84.
30. **Tamm, I., and F. L. Horsfall, Jr.** 1953. Fractional dilution procedure for precise titration of hemagglutinating viruses and hemagglutination-inhibiting antibodies. J. Immunol. **70**:253–259.
31. **Valentine, F.** 1971. Lymphocyte transformation: the proliferation of human blood lymphocytes stimulated by antigen in vitro, p. 443–454. *In* B. R. Bloom and P. R. Glade (ed.), In vitro methods in cell-mediated immunity. Academic Press Inc., New York.
32. **Virelizier, J. L.** 1975. Host defense against influenza virus. The role of antihemagglutinin antibody. J. Immunol. **115**:434–439.
33. **Virelizier, J. L., R. Postlethwaite, G. C. Schild, and A. C. Allison.** 1974. Antibody responses to antigenic determinants of influenza virus hemagglutinin. I. Thymus dependence and antibody formation and thymus independence of immunological memory. J. Exp. Med. **140**:1559–1570.
34. **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.
35. **Warrent, L.** 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. **234**:1971–1975.
36. **Webster, R. G., and W. G. Laver.** 1967. Preparation properties of antibody directed specifically against the neuraminidase of influenza virus. J. Immunol. **101**:49–60.
37. **Webster, R. G., W. G. Laver, and E. D. Kilbourne.** 1968. Reactions of antibodies with surface antigens of influenza virus. J. Gen. Virol. **3**:315–326.
38. **Wyde, P. R., R. B. Couch, B. F. Mackler, T. R. Cate, and B. M. Levy.** 1977. Effects of low- and high-passage influenza virus infection in normal and nude mice. Infect. Immun. **15**:221–229.