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Hemagglutinin-Specific Antibody Responses in Immunoglobulin G, A, and M Isotypes as Measured by Enzyme-Linked Immunosorbent Assay After Primary or Secondary Infection of Humans with Influenza A Virus

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The isotype-specific antibody responses to purified hemagglutinin of adults undergoing either primary or secondary infection with an influenza A virus were characterized by using an enzyme-linked immunosorbent assay. Twenty-eight military recruits undergoing primary infection with A/USSR/92/77 (H1N1)-like virus had serum antibody rises in the immunoglobulin M (IgM) (86%), IgG (100%), and IgA (96%) isotypes. In contrast, 19 adult volunteers undergoing secondary infection with A/Peking/2/79 (H3N2) wild-type virus had serum antibody titer rises largely restricted to the IgG (68%) and IgA (74%) classes, with only 1 volunteer having a serum IgM antibody titer rise. Nasal wash hemagglutinin-specific antibody responses in the adults undergoing secondary infection were predominantly in the IgA class (74%). There was a correlation between the presence of and the magnitude of nasal wash and serum hemagglutinin-specific IgA antibody responses in these adults. This suggested that there was a common source for the hemagglutinin-specific local IgA antibody and serum IgA antibody produced after infection. The recruits undergoing primary H1N1 influenza virus infection had H1 hemagglutinin-specific enzyme-linked immunosorbent assay antibody in each of the IgA, IgG, and IgM isotypes in their acute-phase serum. However, no role for this cross-reactive antibody in modifying the severity of illness experienced by the recruits could be demonstrated.

The enzyme-linked immunosorbent assay (ELISA) has been used to measure serum and local immunoglobulin A (IgA), IgG, and IgM antibody with hemagglutinin (HA) purified from influenza virus as antigen (20, 21). The antibody response in children undergoing primary infection with an influenza A cold-adapted vaccine virus (19) has been analyzed, and most children developed a serum IgG, IgM, and IgA antibody response. Nasal wash antibodies in the IgA, IgM, and IgG isotypes were also detected. Little information exists on the IgA, IgG, and IgM responses of adults infected with influenza A viruses.

In the present study the IgG, IgA, and IgM antibody response of two groups of adults undergoing either "primary" or "secondary" infection with an influenza A wild-type virus was characterized. The first group consisted of 28 seronegative military recruits (mean age 21.5 years) who were naturally infected in early 1978 with an A/USSR/92/77 (H1N1)-like virus during the first season an H1N1 virus had circulated since 1957. The young age of the recruits and their seronegative status indicate that this was their first (i.e., primary) infection with an influenza virus belonging to the H1N1 subtype. The second group of adults was composed of 19 volunteers undergoing secondary infection with an influenza A virus belonging to the H3N2 subtype. These volunteers, who were experimentally infected with A/Peking/2/79 (H3N2) wild-type virus, had low titers (≤1:8) of serum hemagglutination-inhibiting (HAI) antibody to this current H3N2 virus, but had immunological evidence of prior infection with earlier H3N2 viruses. The serum and local antibody responses of these volunteers were measured and compared with the serum responses of the recruits undergoing primary infection and to serum and local responses of children as previously reported (19).

MATERIALS AND METHODS

Clinical specimens. (i) Primary infection. Serum samples were obtained from 28 military recruits who were naturally infected with an A/USSR/92/77-like H1N1 virus during January and February of 1978. The acute phase sera were taken within 3 days after the onset of influenza-like illness, and the convalescent-phase sera were taken 3 weeks later. Oral temperatures were taken at the time the recruits sought medical attention for a respiratory tract infection, and pharyngeal washes were obtained at this time for virus isolation. The presence of infection in each recruit was determined by virus isolation in primary rhesus monkey kidney cells and by detection of a fourfold or greater rise in complement-fixing or HAI antibody in the paired sera. The age of the recruits ranged from 18 to 26 years old (mean age, 21.5 years; 88% ≤22 years old). Each recruit had an HAI antibody titer of ≤1:8 to the A/USSR/92/77 (H1N1) virus in his acute-phase serum sample. The age, serological status, and susceptibility to infection, and illness of these 28 recruits suggested that they were undergoing primary infection with influenza A virus of the H1N1 subtype. Nasal wash specimens were not available from these recruits. Seven additional recruits similarly infected with A/USSR/92/77-like (H1N1) influenza A virus were identified who had antibody titers of >1:8 to A/New Jersey/1/76 (H1N1) antigen in their acute-phase sera presumably acquired by vaccination during the National Influenza Immunization Program in 1976 and 1977 or by natural infection with swine influenza virus. Data from these recruits were analyzed separately.

(ii) Secondary infection. Nineteen adult volunteers who were recruited with informed consent from the community of Baltimore, Md., were experimentally infected with a cloned suspension of A/Peking/2/79 (H3N2) wild-type virus. This virus, which was kindly provided by Alan Kendal (Centers for Disease Control, Atlanta, Ga.), was passaged three times in the allantoic cavity of eggs and six times in calf kidney cells, including a plaque-to-plaque passage, before being grown in the allantoic cavity of specific-pathogen-free eggs (Spafas Inc., Norwich, Conn.) by Louis Potash (Flow Laboratories, McLean, Va.). The details of the virological and clinical aspects of this study will be the topic of a separate report. Briefly, the virus was administered intranasally to 23 volunteers with pre-inoculation HAI antibody titers of ≤1:8 against A/Peking/2/79 (H3N2) at doses of $10^{4.0}$, $10^{5.0}$, or $10^{6.0}$ 50% tissue culture infective doses. Serum samples and nasal wash specimens were obtained before infection and at weekly intervals for 4 weeks after infection. Nasal wash specimens were collected and concentrated as previously described (27). Data obtained from 19 volunteers who were infected as determined by virus shedding or the development of an immunological response (or both) are included in this report. Of the 19 volunteers, 3 developed a minor illness, 18 shed virus, and 17 developed an immunological response. Eighteen of the volunteers had a pre-inoculation HAI serum antibody titer of ≥1:8 to the A/Aichi/2/68 (H3N2) prototype virus (geometric mean titer, 1:37), and the 19th volunteer had pre-inoculation nasal wash antibody to H3 HA. The presence of preexisting antibody to the prototype H3N2 virus indicates that these volunteers were undergoing at least a second infection with an influenza A virus belonging to the H3N2 subtype.

HAI assays. The antigens used in the standard microtiter HAI assays (8) were (i) A/USSR/92/77 (H1N1) split virus vaccine (Parke-Davis & Co., Hunt Valley, Md.), (ii) A/New Jersey/1/76 (H1N1) split virus vaccine (Parke-Davis), (iii) reassortant whole virus possessing the A/Aichi/2/68 (H3N2) HA and the A/Equine/1/Prague/56 neuraminidase, and (iv) A/Peking/2/79 (H3N2) virus grown in chicken embryo allantoic fluid.

ELISA. HA was purified from the A/USSR/90/77 (H1N1) wild-type virus and the X73 reassortant virus of A/Bangkok/1/79 (H3N2) as previously described (25). The HA of this virus is closely related to the HA of the concurrently isolated A/Peking/2/79 (H3N2) virus (personal communication, Alan Kendal). The HA preparations were more than 95% free of other proteins as determined by polyacrylamide gel electrophoresis. The ELISA was performed as described previously (20) with a ladder of reagents, from the solid phase (polystyrene plate, Immulon I; Dynatech Laboratories, Alexandria, Va.) up, consisting of the following: (i) purified HA; (ii) human serum or nasal wash concentrate; (iii) rabbit anti-human IgA, IgG, or IgM; (iv) goat anti-rabbit IgG serum conjugated to alkaline phosphatase; and (v) substrate. The chromogen produced was measured by absorbance at 405 nm. The endpoint was taken as the highest dilution of antibody producing an absorbance of ≥0.1 if the reading in the antigen-containing well was at least twice that in the antigen-free well.

RESULTS

Antibody responses during primary and secondary infection. After primary infection with H1N1 influenza virus, each of the 28 recruits had a rise in HA-specific serum IgG antibody (Table 1); 27 recruits had a significant rise in serum IgA antibody, and 24 had a rise in serum IgM antibody. Thus, most adults undergoing primary infection with an H1N1 influenza A virus had HA-specific antibody responses in each of the IgA, IgG, and IgM isotypes. This response is similar to that expected for a primary response and is comparable to that previously reported in children undergoing primary infection (19). These latter findings are included in Table 1 for comparison.

In contrast, adult volunteers undergoing a secondary H3N2 infection had fewer antibody responses in the IgA (14 of 19) and IgG (13 of 19) isotypes, whereas only one individual had a rise in the IgM antibody isotype. By Chi-square analysis with the Yates correction the difference in frequency of IgM responses in adults with primary infection (24 of 28) compared with the frequency of IgM responses in adults with secondary infection (1 of 19) is highly significant ($\chi^2 = 26$, $P > 0.001$). As expected there was a significant increase in the geometric mean titer of each class of serum antibody in the recruits

TABLE 1. Frequency of influenza A virus HA-specific antibody response in the IgA, IgG, and IgM classes as measured by ELISA

Specimen	Antibody isotype	Number of fourfold or greater rises/number infected (%)		
		Primary infection[a] in adults	Secondary infection[b] in adults	Primary infection[c] in children
Serum	IgA	27/28 (96)	14/19 (74)	13/17 (76)
	IgG	28/28 (100)	13/19 (68)	17/17 (100)
	IgM	24/28 (86)	1/19 (5)	16/17 (94)
Nasal wash	IgA	ND[d]	14/19 (74)	14/17 (82)
	IgG	ND	3/19 (16)	9/17 (53)
	IgM	ND	0/19 (0)	13/17 (76)

[a] Military recruits with <1:8 HAI antibody to H1N1 influenza virus who were naturally infected with A/USSR/92/77 (H1N1)-like virus during the first A/USSR/92/77 outbreak (1977 and 1978).

[b] Adults with preexisting antibody to H3N2 virus who were experimentally infected with A/Peking/2/79 (H3N2) wild-type virus.

[c] Children lacking preexisting HAI antibody or HA-specific antibody, as measured by ELISA, who were experimentally infected with an H1N1 or H3N2 cold-adapted vaccine virus (19).

[d] ND, Not done.

undergoing primary infection, whereas in the volunteers undergoing secondary infection the geometric mean titers rose only in the IgA and IgG classes (Table 2). Although the assay was not standardized to allow direct comparisons of antibody quantity, comparisons of the magnitude of change in titer among the antibody isotypes within each of the groups can be made. During either primary or secondary infection in adults the log fold rises in HA-specific IgA and IgG antibodies were comparable.

The nasal wash antibody response after secondary infection in adults was found to be predominantly IgA (Table 1). This is in contrast to primary infection in children, in whom a majority also had IgM and IgG responses (19).

Correlation between serum and nasal wash IgA antibody response. We noted that after primary infection children with a local IgA antibody response also had a serum IgA antibody response (19). We sought to determine whether this correlation would also be seen after secondary infection in adults. In all but one instance (a child with primary infection), persons with a local IgA response also had a serum IgA response. Among 19 adults with secondary infection, 5 had no IgA antibody response and 14 had both serum and local IgA antibody responses. Among 17 children with primary infection, 1 child had a local IgA response only, 3 had no response, and 13 had both local and serum response. The rise in local antibody was significantly correlated with a rise in serum antibody ($P < 0.01$, Fisher exact test). A similar correlation was not observed for IgG, for which a large number of serum responses were observed in the absence of a local response.

These findings, which demonstrated that there was a correlation between the presence of a local and serum IgA antibody response, suggested that there might be a correlation between the magnitude of the responses as well. There was a significant correlation between the peak titer of IgA in the serum and nasal wash specimens (rho = 0.53; $P \leq 0.05$) (Fig. 1).

Absence of a correlation between the level of preexisting HA-specific ELISA antibody and the level of the febrile response to primary infection in

TABLE 2. Magnitude of influenza A virus HA-specific serum antibody response in the IgG, IgM, and IgA classes as measured by ELISA

Infection (*n* adults)	Serum	Reciprocal of mean $\log_2$ (±SEM) ELISA antibody titer in the indicated immunoglobulin isotype[a]		
		IgA	IgG	IgM
Primary (28)	Acute	8.1 ± 0.29	9.1 ± 0.31	9.1 ± 0.28
	3 wk	12.0 ± 0.25	13.1 ± 0.30	12.4 ± 0.29
Secondary (19)	Preinfection	7.0 ± 0.34	10.1 ± 0.38	8.4 ± 0.31
	3 wk	9.6 ± 0.58	12.2 ± 0.44	8.5 ± 0.27

[a] Values were statistically significantly different ($P < 0.001$, Student's *t* test), except for results with IgM in the secondary infection.

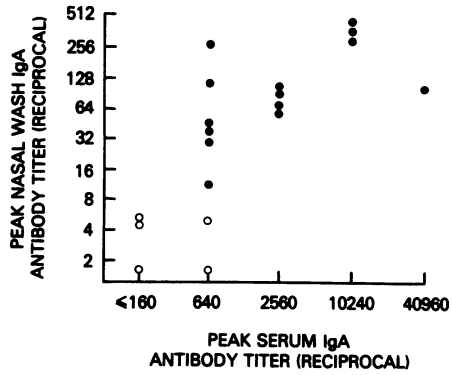

FIG. 1. Peak ELISA titer of serum HA-specific IgA antibody achieved 2 to 4 weeks after infection is plotted against the peak titer of nasal wash HA-specific IgA antibody. Solid circles represent the secondarily infected adults with ≥4-fold rises in IgA antibody titers. For these 14 responders, serum and nasal wash peak titers are significantly correlated (rho = 0.53; $P = 0.05$). Peak values for the five volunteers who failed to have IgA titer rises are represented by open circles.

adults. With the HA-specific ELISA, antibody to the H1N1 HA was previously detected in persons with no known exposure to a virus bearing an H1N1 HA (20). In the present study there was a substantial titer of IgA, IgG, and IgM HA-specific antibody in the acute serum of every recruit undergoing primary infection with an H1N1 virus. In each of the isotypes the titer of the preexisting HA-specific antibody ranged from 1:100 to 1:6,400. In contrast, young seronegative children did not have this preexisting antibody (19). It is possible that this antibody could play a role in modifying the illness experienced by infected persons. To evaluate this possibility the magnitude of the febrile response at the time the recruit sought medical attention (all recruits were shedding virus at this time) was compared with the level of H1 HA-specific IgG antibody (and separately IgA and IgM) in his acute serum. To assess whether this method of analysis could reveal a protective effect of preexisting antibody, the same correlation was made for the seven recruits who had HAI antibody to A/New Jersey/1/76 (H1N1) antigen in their acute-phase serum. This correlation was attempted because antibody to the A/New Jersey/1/76 (H1N1) virus derived by vaccination or infection might be expected to be partially protective against illness caused by the related A/USSR/92/77 (H1N1) virus. Among the seven recruits with HAI antibody to the A/New Jersey/76 virus, temperatures at the time of virus culture were inversely correlated with the reciprocal of their preexisting ELISA IgG antibody titer to A/USSR/92/77 HA. Such a relationship was not detected in the IgG, IgA, or IgM antibody isotypes for the 28 A/New Jersey/1/76 HAI antibody-negative recruits (Fig. 2) (only IgG data presented).

DISCUSSION

The 28 recruits who had primary infection with an influenza virus of the H1N1 subtype developed antibody responses in each of the IgM, IgG, and IgA isotypes. This is typical of the immunological response after primary exposure to a new antigen (15) and resembles that described after infection with other viruses (5, 7, 13, 16, 17, 22–24, 33). In contrast, 18 of the 19 adult volunteers who had a secondary infection with an H3N2 influenza A virus had antibody responses expected for persons reexposed to the same antigen, since they failed to develop a significant IgM antibody response. Again, this is consistent with the pattern expected for reinfection with homologous viruses in which IgM responses are often absent or contribute a relatively small proportion of total antibody (3, 13, 14, 17, 28, 34). The lack of detectable IgM response during secondary infection in our subjects was unlikely to be caused by technical problems such as the presence of specific high-titer competing IgG antibody that could block all of the sites on the bound HA. This was felt to be the case since the recruits undergoing primary infection developed high titers of IgG antibody

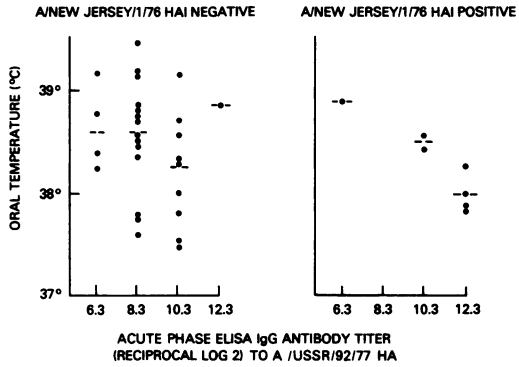

FIG. 2. Oral temperature at the time of acute A/USSR/92/77 (H1N1)-like influenza virus infection is plotted against the acute phase serum titer of IgG antibody specific for HA of the A/USSR/92/77 (H1N1) virus. Values for the 7 recruits with ≥1:16 HAI antibody to A/New Jersey/1/76 (H1N1) antigen and values for the 28 recruits with ≤1:8 HAI antibody are shown separately. No correlation between temperature and antibody titer was found for the 28 seronegative recruits (rho = −0.01; $P > 0.20$); for the 7 seropositive recruits there is a significant (inverse) correlation (rho = −0.96; $P < 0.01$).

to HA, and the increase in IgM antibody was readily detected in the same serum pairs. Furthermore, previous work in this system had shown that the removal of IgG from the serum by adsorption to *Staphlococcus* sp. A protein-Sepharose did not affect the HA-specific IgM titer (20).

The secondarily infected adults described in this report did not have a classic secondary infection in which there was reinfection with homologous virus, but were infected with a heterologous H3N2 virus that had undergone antigenic drift. Presumably this strain differed from the earlier infecting H3N2 strain(s) in one or more of the four major HA epitopes defined by monoclonal antibodies and by sequence analysis of naturally occurring drift strains (30, 32). It was uncertain whether the magnitude of change that occurs during antigenic drift in an influenza A virus was sufficient to give rise to an IgM antibody response directed against the new epitopes on the HA of the drift strain. In the present report we found that, although adults can recognize H1N1 HA as new (i.e., a primary response is generated), the changes in the HA molecule of the A/Peking/2/79 H3N2 virus were not of a sufficient magnitude to elicit a detectable IgM response. Previous data describing IgM antibody responses after influenza virus infection conflict since some authors describe an IgM response after secondary infection, whereas others do not (1, 6, 29, 31). The reasons for these differences remain undefined.

The presence of specific local antibody in the upper respiratory tract (secretory IgA) is thought to be a major determinant of resistance to infection with influenza and other respiratory viruses (4, 9–12, 18, 26). However, measurement of local antibody is time consuming and difficult. It has been documented previously that those persons undergoing primary infection with an influenza A virus who developed a local IgA antibody response also developed a systemic IgA antibody response (19). These observations have been extended by the current study in which the same correlation is observed in persons undergoing secondary infection. Furthermore, the magnitude of the local response correlates with that of the systemic response. Considered together these results would suggest that local and serum IgA HA-specific antibody have the same source, i.e., both are derived from mucosal plasma cells producing IgA antibody. The presence of HA-specific IgA antibody in the serum could result from locally produced antibody that escapes secretion through epithelial cells and gains access to the systemic circulation via the lymphatic or venous drainage of the mucosa. This suggests that, after acute infection, a serum IgA response could be used as an indication of the presence of a local IgA response.

The presence of cross-reactive antibody in persons who are unlikely to have had prior exposure to a virus subtype has been noted previously (20). We have confirmed that antibody measurable by ELISA in each of the isotypes is present in the acute serum of each of our recruits even though they have no prior exposure to H1N1 viruses. This antibody may be reactive with conserved portions of the HA molecule present on both the A/USSR/92/77 HA and on the HA of other subtypes to which the persons have had previous exposure. Alternatively, it may be a cross-reactive antibody induced by noninfluenza antigens. It is clear that this antibody is absent in the sera of children who have had experience with only one of the H1N1 or H3N2 viruses (19). The data from the present study suggest that this antibody does not confer resistance to illness since there is no correlation between the level of illness observed and the quantity of cross-reactive antibody present. We want to emphasize that this observation is preliminary and is based on a single temperature recorded at the time of acute illness. However, similar data from an experimental challenge also failed to indicate any effect of this cross-reactive antibody on the course of infection (2, 20). The origin and possible biological significance of this antibody are under further investigation.

The findings from the present study also have negative implications for the use of an anti-influenza IgM assay to rapidly diagnose the presence of acute influenza A virus infections. First, an assay that relies on detection of IgM antibody reactive with the HA molecule will be positive in all adults due to the presence of cross-reactive antibody. Second, persons undergoing secondary infection within a subtype often fail to develop a specific IgM antibody response. These limitations suggest that methods to detect viral antigens or nucleic acids rather than acute-phase IgM antibody titer will be needed to rapidly document acute influenza A infection in humans.

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LITERATURE CITED

1. **Bucher, Y. I., R. B. Heath, J. V. Collins, and J. R. Patterson.** 1977. Serum IgM antibody and influenza A infection. J. Clin. Pathol. **30**:723–727.
2. **Chanock, R. M., and B. R. Murphy.** 1980. Use of temperature-sensitive and cold-adapted mutant viruses in immunoprophylaxis of acute respiratory tract disease. Rev. Infect. Dis. **3**:421–432.

3. **Corthier, G., B. Charley, and J. Franz.** 1980. Local immunity in the pig respiratory tract. Ann Virol. **131E**:355–363.
4. **Couch, R. B., R. G. Douglas, Jr., R. Rossen, and J. A. Kasel.** 1969. Role of secretory antibody in influenza, p. 93–112. *In* D. H. Dayton, Jr., P. A. Small, Jr., R. M. Chanock, H. E. Kaufman, and T. B. Tomasi, Jr. (ed.), The secretory immunologic system. U.S. Government Printing Office, Washington, D.C.
5. **Cukor, G., N. A. Nowak, N. R. Blacklow.** 1982. Immunoglobulin M responses to the norwalk virus of gastroenteritis. Infect. Immun. **37**:463–468.
6. **Daugharty, H., M. L. Davis, and H. S. Kaye.** 1972. Immunoglobulin class of influenza antibodies investigated by radioimmunoassay (RIA). J. Immunol. **109**:849–856.
7. **Dorries, R., and V. terMeulen.** 1980. Detection of enterovirus specific IgG and IgM antibodies in humans by an indirect solidphase radioimmunoassay. Med. Microbiol. Immunol. **168**:159–171.
8. **Dowdle, W. A., A. P. Kendal, and G. R. Nobel.** 1979. Influenza viruses, p. 585–609. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.
9. **Fazekas de St. Groth, S.** 1950. Influenza: a study in mice. Lancet **i**:1101–1105.
10. **Fazekas de St. Groth, S., and M. Donnelley.** 1950. Studies in experimental immunology of influenza IV. The protective effects of active immunization. Aust. J. Exp. Biol. Med. Sci. **28**:62–75.
11. **Francis, T., Jr.** 1943. A rational for studies in the control of epidemic influenza. Science **97**:229–235.
12. **Francis, T., Jr., and I. J. Brightman.** 1941. Virus inactivating capacity of nasal secretions in the acute and convalescent stages of influenza. Proc. Soc. Exp. Biol. Med. **48**:116–117.
13. **Gonchoroff, N. J., A. P. Kendal, D. J. Phillips, and C. B. Reimer.** 1982. Immunoglobulin M and G antibody response to type- and subtype-specific antigens after primary and secondary exposures of mice to influenza A viruses. Infect. Immun. **36**:510–517.
14. **Kalimo, K. O. K., R. J. Marttila, K. Granfors, and M. K. Viljanen.** 1977. Solid-phase radioimmunoassay of human immunoglobulin M and immunoglobulin G antibodies against herpes simplex virus type 1 capsid, envelope, and excreted antigens. Infect. Immun. **15**:883–889.
15. **Kongsham, P. A., D. Hawkins, and J. Shuster.** 1976. The biology of the immune response, p. 1–64. *In* S. O. Freedman and P. Gold (ed.), Clinical immunology, 2nd ed. Harper & Row Publishers, Hagerstown, Md.
16. **Lerner, A. M., M. J. Shippey, and L. R. Crane.** 1974. Serologic responses to herpes simplex virus in rabbits: complement-requiring neutralizing, conventional neutralizing, and passive hemagglutinating antibodies. J. Infect. Dis. **129**:623–636.
17. **Linnemann, C. C., M. E. Hegg, T. C. Rotte, J. P. Phair, and G. M. Schiff.** 1973. Measles IgM response during reinfection of previously vaccinated children. J. Pediatr. **82**:798–801.
18. **Mills, J., H. L. S. Knopf, J. Vankirk, and R. M. Chanock.** 1969. Significance of local respiratory tract antibody to respiratory synctial virus, p. 149–165. *In* D. H. Dayton, Jr., P. A. Small, Jr., R. M. Chanock, H. E. Kaufman, and T. B. Tomasi, Jr. (ed.), The secretory immunologic system. U.S. Government Printing Office, Washington, D.C.
19. **Murphy, B. R., D. L. Nelson, P. F. Wright, E. L. Tierney, M. A. Phelan, and R. M. Chanock.** 1982. Secretory and systemic immunological response in children infected with live attenuated influenza A virus vaccines. Infect. Immun. **36**:1102–1108.
20. **Murphy, B. R., M. A. Phelan, D. L. Nelson, R. Yarchoan, E. L. Tierney, D. W. Alling, and R. M. Chanock.** 1981. Hemagglutinin-specific enzyme-linked immunosorbent assay for antibodies to influenza A and B viruses. J. Clin. Microbiol. **13**:554–560.
21. **Murphy, B. R., E. L. Tierney, B. A. Barbour, R. H. Yolken, D. W. Alling, H. P. Holley, Jr., R. E. Mayner, and R. M. Chanock.** 1980. Use of the enzyme-linked immunosorbent assay to detect serum antibody responses of volunteers who received attenuated influenza A virus vaccines. Infect. Immun. **29**:342–347.
22. **Ogra, P. L., and D. T. Karzon.** 1969. Poliovirus antibody response in serum and nasal secretions following intranasal innoculation with inactivated poliovaccine. J. Immunol. **109**:15–23.
23. **Ogra, P. L., D. T. Karzon, F. Righthand, and M. MacGillirray.** 1968. Immunoglobulin response in serum and secretions after immunization with live and inactivated polio vaccine and natural infection. N. Engl. J. Med. **279**:893–900.
24. **Ogra, P. L., D. Kerr-Grant, G. Umana, J. Dzierba, and D. Weintraub.** 1971. Antibody response in serum and nasopharynx after naturally acquired and vaccine induced infection with rubella virus. N. Engl. J. Med. **285**:1333–1339.
25. **Phelan, M. A., R. E. Mayner, D. J. Bucher, and F. A. Ennis.** 1980. Purification of influenza virus glycoproteins for the preparation and standardization of immunological potency testing reagents. J. Biol. Stand. **8**:233–242.
26. **Plotkin, S. A., J. D. Farquhar, and P. L. Ogra.** 1973. Immunologic properties of RA 27/3 rubella virus vaccine a comparison with strains presently licensed in the United States. J. Am. Med. Assoc. **225**:585–590.
27. **Richman, D. D., B. R. Murphy, R. M. Chanock, J. M. Gwaltney, R. G. Douglas, R. F. Betts, N. R. Blacklow, F. B. Rose, T. A. Parrino, M. M. Levine, and E. S. Caplan.** 1976. Temperature-sensitive mutants of influenza A virus. XII. Safety, antigenicity, transmissibility and efficacy of influenza A/Udorn/72-ts-1E recombinant viruses in human adults. J. Infect. Dis. **134**:585–594.
28. **Schluederberg, O., and S. Karelitz.** 1965. Suppression of measles 19S antibody formation as evidence of immunity. Primary immune response masked by maternal antibody. J. Am. Med. Assoc. **191**:1064–1066.
29. **Urquhart, G. E. D.** 1974. Serum IgM and IgA responses in influenza A infections. J. Clin. Pathol. **27**:198–201.
30. **Verhoeyen, M., R. Fang, W. M. Jou, R. Devos, D. Huylebroeck, E. Saman, and W. Fiers.** 1980. Antigenic drift between the hemagglutinin of the Hong Kong influenza strains A/Aichi/2/68 and A/Victoria/3/75. Nature (London) **286**:771–776.
31. **Waldman, R. H., J. A. Kasel, R. H. Alford, and J. J. Mann.** 1967. Immunoglobulin classes of serum neutralizing antibody formed in response to infection with human A2 influenza virus. Proc. Soc. Exp. Biol. Med. **125**:316–321.
32. **Webster, R. G., W. G. Laver, G. M. Air, and G. C. Schild.** 1982. Molecular mechanisms of variation in influenza viruses. Nature (London) **296**:115–121.
33. **Welliver, R. C., T. N. Kaul, T. I. Putnam, M. Sun, K. Riddlesberger, and P. L. Ogra.** 1980. The antibody response to primary and secondary infection with respiratory synctial virus: Kinetics of class-specific responses. J. Pediatr. **96**:808–813.
34. **Zee, Y. C., J. W. Osebald, and W. M. Dotson.** 1979. Antibody responses and interferon titers in the respiratory tracts of mice after aerosolized exposure to influenza virus. Infect. Immun. **25**:202–207.