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Regional T- and B-Cell Responses in Influenza-Infected Ferrets

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Ferrets were infected with A/Port Chalmers/73 influenza virus and the T- and B-cell responses in the spleen, in lymph nodes draining the upper and lower respiratory tract, and in lung washings were examined in vitro. Lymphocyte responses were measured by using a hemolytic plaque assay for B cells and a proliferation assay for T cells. Virus and antibody levels were measured in respiratory tract washings, and antibody titers were measured in sera from infected animals. Individual B cells secreting specific antibody to A/Port Chalmers/73 virus were detected in regional lymph node and spleen preparations as early as 3 days and as late as 43 days after infection. T-cell assays showed an in vitro response of lymph node cells to A/Port Chalmers/73 virus from day 6 to day 43. Virus was isolated from the respiratory tract up to 7 days after infection. Serum hemagglutination-inhibiting antibody was first detectable on day 6, with maximum titers reached by day 10. These results demonstrated that antibody production and a cellular immune responses were detectable at regional sites at a time when virus was still present and before serum antibody was measured.

Influenza infection of humans is a localized respiratory tract infection from which recovery is usually complete in 1 to 2 weeks. The roles of humoral and cellular immunity in this recovery are not clear. Immune responses to influenza virus infections have usually been studied by measuring antibody production. Antibody to the virus can be detected in serum and respiratory tract secretions after infection or immunization with influenza virus. Although studies of this kind have indicated that specific antibody may be detected locally in the respiratory tract (21), they do not give a specific site for this production. Another approach has been to quantitate the total number of B cells in the respiratory tract and lung tissue (1, 24).

The hemolytic plaque assay of Jerne et al. (15) has been adapted to the measurement of B cells secreting antibody to influenza viruses (16a). We have utilized this sensitive technique to examine regional lymph node and splenic responses of ferrets infected with A/Port Chalmers/1/73 influenza virus, because the disease in ferrets is similar to that in humans (19). Lymphocytes from these animals were also tested in a lymphocyte proliferation assay to detect the presence of sensitized T cells. B cells producing specific antibody were detected in regional lymph nodes and spleen at 2 to 3 days before serum hemagglutination-inhibiting antibody was detected. T-cell responses occurred slightly later than B-cell responses, but were maintained for longer periods of time.

MATERIALS AND METHODS

Influenza viruses. Influenza virus A/Port Chalmers/1/73 (H3N2) (A/Pt Ch/73) was obtained from virus pools maintained at the Bureau of Biologics and was grown in embryonated hens eggs by standard procedures. A Formalin-inactivated, zonally purified preparation of the high-yielding recombinant virus A/Pt Ch/73 (MRC-11) (H3N2) was kindly supplied by Merrell-National Laboratories, Swiftwater, Pa.

Ferrets. Young adult male ferrets (1 to 1.4 kg) were obtained from Marshall Research Animals Inc., North Rose, N.Y. Animals were immunized with canine distemper virus vaccine before use.

Antibody titrations. Hemagglutination-inhibiting (HI) antibodies in sera and respiratory tract washings were titrated in a standard microassay after treatment with 1.11×10^{-2} M potassium periodate and glycerol saline (18). Because of low levels of residual nonspecific inhibitors, serum HI antibody titrations were performed with starting dilutions of 1:20. Respiratory tract washings were tested at a starting dilution of 1:10. Infectivity neutralization tests of sera and nasal and lung washings were performed by inoculation of eggs with serial dilutions of each specimen mixed with 100 50% egg infective doses (EID_{50}) of A/Pt Ch/73 virus.

Measurement of virus titer in respiratory tract specimens. Infectivity titers of unconcentrated nasal and lung washings and homogenates of lung tissue were determined by titration in eggs. End points were calculated by the method of Spearman-Karber (7) and

expressed as EID_{50} per 0.2 ml.

Preparation of lymphocyte suspensions. Lymph nodes and spleens were dissociated in a similar way. Capsular and fibrous material was dissected away, and the tissue was initially minced with a scalpel and placed in 2 to 4 ml of Hanks saline at 4°C. Cell suspensions were then prepared by repeatedly aspirating and expelling the tissue suspension from a 1-ml syringe. Debris was removed by aspiration, and samples were removed for cell counts. Viability counts were performed by using trypan blue after lysis of erythrocytes in ammonium chloride buffer (2); viability was usually 90% or more.

Cells were obtained from bronchial washings by centrifugation of the unconcentrated washings. The pelleted cells were suspended in 1 ml of Hanks saline and counted as described above.

Hemolytic plaque assay. The hemolytic plaque assay was carried out as described previously (16a). Briefly, 3-week-old sheep erythrocytes (SRBC) were treated with a suitable dilution of concentrated A/Pt Ch/73 influenza virus vaccine in the presence of potassium periodate. The vaccine dilution was determined by testing of virus-coated SRBC in a single radial hemolysis assay using mouse anti-A/Pt Ch/73 virus serum (23). Washed, treated SRBC were suspended in an equal volume of Veronal-buffered saline, pH 7.2. Plaque assays were performed by using 0.2 ml of SRBC and 0.1 ml of lymphocyte suspension added to 2 ml of nutrient agarose. Thin layers of agarose-cell suspensions were prepared as described previously (16a). Cultures were incubated for 60 to 90 min at 37°C in a humidified atmosphere of 5% CO_2 in air before diluted guinea pig complement was added. After a further 30-min incubation the complement was removed, and the plaques were counted with a dissecting microscope. The number of plaque-forming cells (PFC) per 10^6 viable lymphocytes was calculated; results from three to five animals at each time point were used to calculate the mean and standard error of the mean for each point.

Lymphocyte proliferation assay. Samples of the lymphocyte suspensions used in hemolytic plaque assays were also tested in a proliferation assay (G. M. Butchko, W. J. Martin, and F. A. Ennis, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, S229, p. 317; J. Immunol., in press). Remaining erythrocytes were lysed by treatment with ammonium chloride buffer, and lymphocytes were suspended to 2×10^6 viable cells per ml in RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum and 2×10^{-4} M 2-mercaptoethanol. Volumes of 0.2 ml of lymphocyte suspension from either A/Pt Ch/73 virus-infected or control uninfected ferrets were added to wells of microtiter plates (Microtest II; Falcon Plastics, Oxnard, Calif.). Live A/Pt Ch/73 virus was added in a volume of 10 μl to quadruplicate samples of normal and immune lymphocytes. In some experiments, concanavalin A and *Escherichia coli* lipopolysaccharide in a range of dilutions were used as T- and B-cell mitogens. Cultures were incubated for 44 h at 37°C in a 5% CO_2–95% air atmosphere. At this time, 1 μCi of [^{3}H]-leucine (specific activity, 55 Ci/mmol; Amersham Corp.) was added per well. After 4 h the cells were collected onto glass fiber filter strips by using a MASH II harvester (Microbiological Associates, Bethesda, Md.), and the incorporated radioactivity was determined by scintillation counting.

A stimulation index (SI) for each set of cultures was calculated from the mean counts per minute of four replicate cultures containing virus or mitogen divided by the mean counts per minute incorporated in the unstimulated quadruplicate cultures of the same cell suspension. In all experimental groups, the ranges of the SIs observed in quadruplicate samples were within 10% of the mean value shown.

Fractionation of lymphocyte suspensions. To determine the class of lymphocytes responding to A/Pt Ch/73 virus in proliferative assays, lymph node and spleen cell suspensions were depleted of B cells by passage through nylon fiber columns by the method of Julius et al. (16). The nonadherent cells showed an increased response to concanavalin A and a decreased response to *E. coli* lipopolysaccharide and will be referred to as the T-cell-enriched population.

Experimental design. Groups of ferrets were infected under light anesthesia (sodium pentobarbital; Veterinary Laboratories, Inc., Lenexa, Kans.) by intranasal inoculation with 10^4 to 10^5 EID_{50} of A/Pt Ch/73 virus. At frequent intervals nasal washings were collected (20), and the animals were exsanguinated under anesthesia. Lymph nodes were removed from the sublingual and peritracheal regions. The lungs were then excised with attached trachea; lung washings were collected by instillation and aspiration of 30 ml of phosphate-buffered saline using a 50-ml syringe attached to the trachea. Any obvious periaortic lymph nodes were dissected out, and a number of samples of lung tissue were removed for virus isolation. Sections of spleen tissue were collected. Suspensions of lymphocytes were prepared from the lymph node and spleen preparations; nasal and lung washings and homogenized lung tissue were titrated for infectious virus; sera and concentrated nasal and lung washings were tested for HI antibody; and the protein concentration of concentrated nasal and lung washings was determined by using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.).

Concentration of respiratory tract washings. Nasal washings (7 to 10 ml) and centrifuged lung washings (20 to 30 ml) were concentrated to a final volume of 1 to 1.5 ml by using an Amicon MMC filtration unit with YM-10 membranes (Amicon Corp., Lexington, Mass.). Concentrated specimens were stored at −70°C.

RESULTS

Virus replication and serum antibody response. Virus was recovered from nasal washings collected at 1 to 7 days after infection, with similar titers of virus being measured in lung homogenates (Fig. 1). Virus titers in unconcentrated lung washings were 10- to 100-fold lower than in nasal washings. Serum HI antibody (>1:20) was first detected at 6 days after infection, reached a peak by day 10, and was maintained at high titers up to day 43. Serum specimens collected at 3 days after infection showed

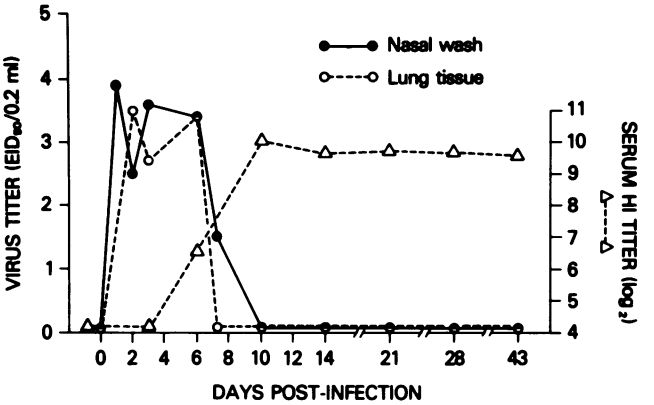

FIG. 1. *Virus replication and serum HI antibody response in A/Pt Ch/73 virus-infected ferrets. Each point is the mean value from three or four animals. Responses were negative for noninfected ferrets.*

no activity in neutralization assays (starting dilution, 1:4).

Respiratory tract washings. The protein content of concentrated nasal washings increased markedly after infection, increasing from less than 0.1 mg/ml to a peak of 0.8 mg/ml on day 3. Levels had returned to preinfection values by day 21 (Fig. 2). None of the concentrated nasal washings contained antibody to A/Pt Ch/73 virus detectable by HI or neutralization tests.

No significant changes in the protein content of concentrated lung washings were observed (Fig. 2). Values were much higher than for nasal washings and ranged from 5 to 8.5 mg/ml. HI antibody was, however, detected in lung washings. Two of four animals had low antibody titers (1:10) at day 14, and all four ferrets tested on day 21 had HI titers of 1:20. This HI activity was apparently not due to nonspecific factors because the lung wash specimens showed inhibitory activity in virus infectivity neutralization tests.

B-cell responses. After infection, marked hyperplasia of the lymph nodes draining the upper and lower respiratory tract was observed. Nodes sometimes had small hemorrhagic spots in the acute stage of infection. PFC responses to A/Pt Ch/73 virus were found in both regional lymph nodes and spleens early after infection (Fig. 3). Thus 26 PFC/10^6 cells were found in sublingual nodes removed 3 days after infection, and 38 PFC/10^6 cells were found on day 10. Similar responses were observed in peritracheal nodes, where a significant response was still measured on days 28 and 43. Periaortic nodes showed a peak response at day 10, with a mean PFC count of 150 per 10^6 cells (range, 14 to 460). Cells collected from lung washings were uniformly negative for PFC at all times except day 10, when high numbers of PFC were found in

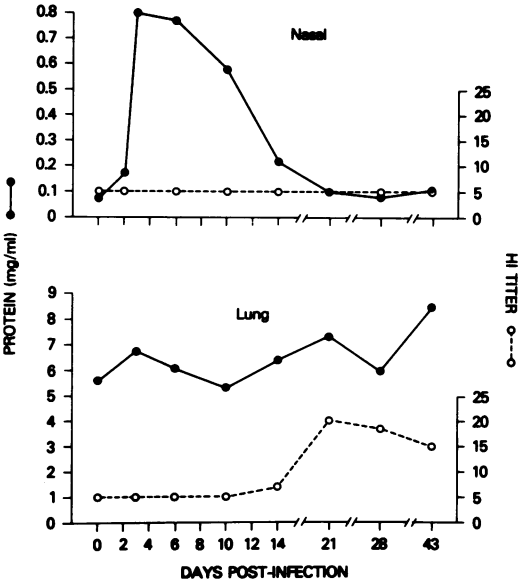

FIG. 2. *Protein and HI antibody responses in respiratory tract washings. Nasal and lung washings were concentrated by membrane filtration. Each point is the mean value from three or four animals. Uninfected animals showed no significant changes in respiratory tract washings.*

cells from three of four ferrets (63, 67, and 215 PFC/10^6 cells). Splenocytes showed a PFC response of 56 PFC/10^6 cells on day 3 and 84/10^6 cells on day 10. A count of 15 PFC/10^6 splenocytes was measured at day 43, but no significant numbers of antibody-forming cells were detected at day 57.

The specificity of the B-cell responses was similar to that reported in murine studies (16a). Thus lymphocytes from A/Pt Ch/73 virus-infected ferrets cross-reacted to a high level with

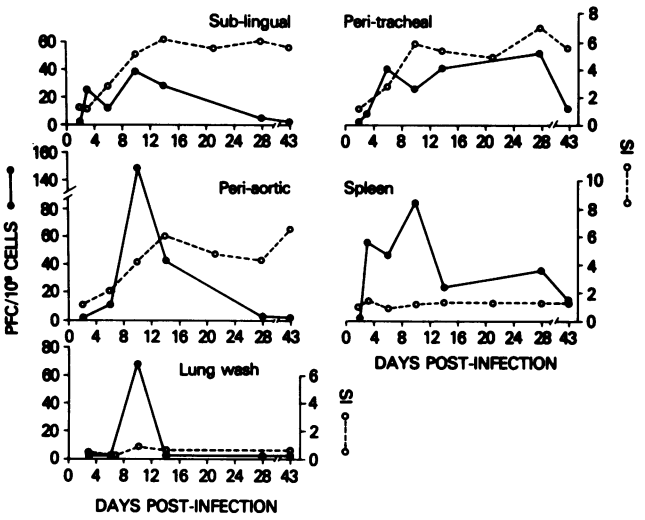

FIG. 3. *B- and T-lymphocyte responses in A/Pt Ch/73 virus-infected ferrets. B-cell responses were measured in a hemolytic plaque assay and are shown as numbers of PFC per 10^6 viable cells (●). T-cell responses were measured in a [^{3}H]leucine-labeled proliferation assay and are shown as SIs (○). Each point is the mean value from three or four animals. Uninfected animals showed no significant PFC response to virus-coated SRBC and gave SI values of less than 1.5.*

SRBC treated with the closely related A/Victoria/75 (H3N2) virus but not with SRBC treated with A/NJ/76 (HswlN1) or B/HK/73 viruses (data not shown).

T-cell responses. Proliferative lymphocyte responses occurred later than the appearance of specific antibody-producing cells, but by day 6 significant levels of in vitro stimulation by A/Pt Ch/73 virus were measured in lymphocytes from regional lymph nodes (Fig. 3). Peak responses, with SI values of approximately 6, were reached by day 14 and continued at this level for the duration of the experiment. In these experiments no significant in vitro stimulation of lung wash or spleen lymphocytes was detected when either SI or differences in counts per minute were used as indexes of response.

Separation of lymph node cells on nylon fiber columns was used to identify the cell population responding in the in vitro proliferation assay. The results from two animals tested at 10 days after infection are shown in Table 1. These results show an enhanced response to both A/Pt Ch/73 virus and the T-cell mitogen concanavalin A and a reduced response to the B-cell mitogen *E. coli* lipopolysaccharide in the nonadherent cell population. These data indicate that the cells responding in vitro to A/Pt Ch/73 virus were T cells.

TABLE 1. *Characterization of cells responding in lymphocyte proliferation assays*

Antigen[a]	SI for:			
	Ferret 173		Ferret 174	
	Untreated[b]	Nonadherent[c]	Untreated	Nonadherent
Concanavalin A	26	4,144	18	5,640
E. coli lipopolysaccharide	16	4	9	1
A/Pt Ch/73 virus	17	202	15	157

[a] Lymphocytes were incubated with one of the following antigens or mitogens: concanavalin A, 3 μg/ml; *E. coli* lipopolysaccharide, 1 μg/ml; or A/Pt Ch/73 virus infectious allantoic fluid, 10 μl/well.

[b] Control (nonfractionated lymph node cell suspension).

[c] Nonadherent refers to cells not retained on nylon wool column.

DISCUSSION

Influenza virus replication occurs in the respiratory tract, with little or no dissemination of virus to other organs. The role of local or regional immune responses to this localized infection may be of great importance and has been examined by previous workers (8, 12–14, 17, 21, 25–27). The techniques employed in these earlier studies, however, have been restricted to examining the respiratory tract secretions for antibody. Other workers have studied the immune responses to localized respiratory infections by enumerating changes in immunoglobulin-secreting lymphocytes in respiratory tract organs.

Thus, Blandford et al. (1) reported an early increase in the numbers of B cells in the lungs of Sendai virus-infected mice but did not show that the B cells in the lungs were producing antibodies specific to Sendai virus. Similar results were obtained by Scott and Walker (24) in influenza virus-infected mice.

Recent studies on cytotoxic T-cell responses to influenza antigens have demonstrated that cytotoxic responses are induced by intranasal infection and by intraperitoneal inoculation of virus (3, 6, 9, 11). The role of the cytotoxic cells in recovery from infection, however, is not fully defined. A limited number of studies of proliferative responses of human lymphocytes to influenza antigens have been presented (4, 5, 22). These earlier results, which give a measure of T-cell memory, have usually shown relatively low levels of in vitro stimulation by influenza viruses.

Many studies have demonstrated the usefulness of ferrets in influenza immunity studies (19). After intranasal infection ferrets demonstrate many of the symptoms of human influenza. Thus, animals show a febrile response and have symptoms of malaise, coryza, and sneezing. Virus may be readily isolated from nasal washings, and antibody may be transiently present in nasal washings (25). The immune responses of ferrets may therefore be of relevance to human responses.

The present study demonstrated that B cells secreting antibody to the infecting influenza virus could be detected in regional lymph nodes and in the spleen as early as 3 days after infection. This was earlier than HI or neutralizing antibodies were detectable in the serum and concurrent with the presence of high titers of virus in nasal washings and lung tissue. The subsequent decline of virus titers may thus be related to the presence of antibody secreted by these B cells, although other effector cells such as T lymphocytes (10) and macrophages may also have a role.

Although a marked increase in the protein content of nasal washings occurred during the acute stage of the infection, no detectable antibody was found in the washings. This is in contrast to previous studies with other virus strains (17, 25). However, antibody was found in concentrated lung washings late in convalescence after virus was no longer detectable in lung tissue and after peak titers of serum antibody had been reached. The class of this antibody is presently unknown. Because of the low antibody titer we are unable to perform velocity gradient ultracentrifugation studies as described previously (25).

No attempt was made in the present study to assign any antibody class to the PFC detected in lymph nodes or spleen. In most hemolytic plaque assays, only immunoglobulin M-secreting PFC are detected in direct assays and an enhancing anti-immunoglobulin G serum must be used to detect immunoglobulin G-secreting cells. With the anti-influenza assay used in this study, however, it has been shown that murine lymphocytes secreting immunoglobulin G antibody can produce plaques in direct assays (16a). Similarly, in experiments using rabbit antiserum to purified ferret immunoglobulin G (kindly purified by J. Finlayson), we have observed little increase in the number of plaques produced in indirect assays compared with direct assays.

A relatively high number of influenza-specific PFC were detected in spleens at a time when PFC were detected in regional lymph nodes. This may indicate that homing of lymphocytes from the respiratory tract to the spleen occurs, as no evidence of viremia in influenza infections of ferrets has been presented. Noninfectious virus or viral antigens could also circulate in the blood stream as either free particles or bound to erythrocytes or lymphocytes (28). The PFC response in the spleen was detectable for 43 days after infection and may explain the maintained serum antibody titers.

Whereas good proliferative responses were found with lymph node cells, splenocytes failed to respond well to either influenza virus or concanavalin A, in contrast to results obtained with murine spleen cells (Butchko et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, S229, p. 317; J. Immunol., in press). The proliferative T-cell response in this study was detected after the appearance of specific antibody-secreting cells and could be measured for a longer time than the B cell response, with no diminution by day 43. These temporal differences may, however, reflect the relative sensitivities of the different assays rather than true biological differences. The biological function of these T cells in the recovery process is not known, because the proliferative assay does not measure functions such as cytotoxicity.

In summary, we have been able to demonstrate T- and B-cell responses to influenza virus early in infection, at a time when virus is still present. The B-cell response can be detected before serum antibody is measured and may indicate that the antibody produced early in infection is rapidly bound to virus antigen and removed from the circulation. This antibody may play a role in the neutralization and elimination of virus.

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LITERATURE CITED

1. **Blandford, G., R. J. R. Cureton, and R. B. Heath.** 1971. Studies of the immune response in Sendai virus infection of mice. J. Med. Microbiol. **4**:351-356.
2. **Boyle, W.** 1968. An extension of the ^{51}Cr-release assay for the estimation of mouse cytotoxins. Transplantation **6**:761-764.
3. **Cambridge, G., J. S. Mackenzie, and D. Keast.** 1976. Cell-mediated immune response to influenza virus infections in mice. Infect. Immun. **13**:36-43.
4. **Cate, T. R., and J. R. Kelly.** 1971. Hong Kong influenza antigen sensitivity and decreased interferon response of peripheral lymphocytes, p. 156-160. Antimicrob. Agents Chemother. 1970.
5. **Cole, P. J., and M. E. Molyneux.** 1975. Lymphocyte reactivity to influenza virus in man. Immunology **29**:749-754.
6. **Doherty, P. C., R. B. Effros, and J. Bennink.** 1977. Heterogeneity of the cytotoxic response of thymus-derived lymphocytes after immunization with influenza viruses. Proc. Natl. Acad. Sci. U.S.A. **74**:1209-1213.
7. **Dougherty, R. M.** 1964. Animal virus titration techniques, p. 183-186. *In* R. J. C. Harris (ed.), Techniques in experimental virology. Academic Press Inc., London.
8. **Downie, J. C., and C. H. Stuart-Harris.** 1970. The production of neutralizing activity in serum and nasal secretion following immunization with influenza B virus. J. Hyg. **68**:233-244.
9. **Effros, R. B., P. C. Doherty, W. Gerhard, and J. Bennink.** 1977. Generation of both cross-reactive and virus-specific T-cell populations after immunization with serologically distinct influenza A viruses. J. Exp. Med. **145**:557-568.
10. **Ennis, F. A., W. J. Martin, and M. W. Verbonitz.** 1977. Hemagglutinin-specific cytotoxic T-cell response during influenza infection. J. Exp. Med. **146**:893-898.
11. **Ennis, F. A., W. J. Martin, M. W. Verbonitz, and G. M. Butchko.** 1977. Specificity studies on cytotoxic thymus-derived lymphocytes reactive with influenza virus-infected cells: evidence for dual recognition of H-2 and viral hemagglutinin antigens. Proc. Natl. Acad. Sci. U.S.A. **74**:3006-3010.
12. **Fedson, D. S., R. V. Fulk, M. A. Huber, M. A. Reisberg, and J. A. Kasel.** 1971. Anti-neuraminidase antibody response in serum and nasal secretions following intranasal or subcutaneous inactivated A2/Hong Kong/68 influenza virus vaccine. J. Immunol. **107**:730-737.
13. **Freestone, D. S., S. Hamilton-Smith, G. C. Schild, R. Buckland, S. Chinn, and D. A. J. Tyrrell.** 1972. Antibody responses and resistance to challenge in volunteers vaccinated with live attenuated, detergent split and oil adjuvant A2/Hong Kong/68 (H3N2) influenza vaccines. J. Hyg. **70**:531-543.
14. **Gwaltney, J. M., W. P. Edmondson, R. Rothenberg, and P. W. White.** 1971. A comparison of subcutaneous, nasal and combined influenza vaccination. I. Antigenicity. Am. J. Epidemiol. **93**:472-479.
15. **Jerne, N. K., C. Henry, A. A. Nordin, H. Fuji, A. M. C. Koros, and I. Lefkovits.** 1974. Plaque forming cells: methodology and theory. Transplant. Rev. **18**:130-191.
16. **Julius, M., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for the isolation of functional thymus-derived lymphocytes. Eur. J. Immunol. **3**:645-648.

16a. **McLaren, C., G. E. Grubbs, and F. A. Ennis.** 1978. Detection of cells producing surface antigen-specific antibody to influenza viruses. J. Clin. Microbiol. **8**:438-444.

17. **McLaren, C., C. W. Potter, and R. Jennings.** 1974. Immunity to influenza in ferrets. X. Intranasal immunization of ferrets with inactivated influenza A virus vaccines. Infect. Immun. **9**:985-990.
18. **Palmer, D. F., M. T. Coleman, W. R. Dowdle, and G. C. Schild.** 1975. Advanced laboratory techniques for influenza diagnosis, p. 25-61. United States Department of Health, Education, and Welfare, Atlanta.
19. **Potter, C. W., C. McLaren, and R. Jennings.** 1975. Assessment of resistance to influenza virus infection in animal models. Dev. Biol. Stand. **28**:307-318.
20. **Potter, C. W., J. S. Oxford, S. L. Shore, C. McLaren, and C. H. Stuart-Harris.** 1972. Immunity to influenza in ferrets. I. Response to live and killed virus. Br. J. Exp. Pathol. **53**:153-167.
21. **Rossen, R. D., J. A. Kasel, and R. B. Couch.** 1971. The secretory immune system: its relation to respiratory viral infection, p. 194-238. *In* J. L. Melnick (ed.), Progress in medical virology. S. Karger, Basel.
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. **Russell, S. M., D. McCahon, and A. S. Beare.** 1975. A single radial haemolysis technique for the measurement of influenza antibody. J. Gen. Virol. **27**:1-10.
24. **Scott, G. H., and J. S. Walker.** 1976. Immunoglobulin-bearing cells in lungs of mice infected with influenza virus. Infect. Immun. **13**:1525-1527.
25. **Shore, S. L., C. W. Potter, and C. McLaren.** 1972. Immunity to influenza in ferrets. IV. Antibody in nasal secretions. J. Infect. Dis. **126**:394-400.
26. **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.
27. **Waldman, R. H., S. H. Wood, E. J. Torres, and P. A. Small.** 1970. Influenza antibody response following aerosol administration of inactivated virus. Am. J. Epidemiol. **91**:575-584.
28. **Wilson, A. B., D. N. Planterose, J. Nagington, J. R. Park, R. D. Barry, and R. R. A. Coombs.** 1976. Influenza A antigens on human lymphocytes *in vitro* and probably *in vivo*. Nature (London) **259**:582-584.