THE JOURNAL OF IMMUNOLOGY

Vol. 118, No. 1, January 1977
Printed in U.S.A.

DEFENSE MECHANISMS AGAINST PRIMARY INFLUENZA VIRUS INFECTION IN MICE

I. The Roles of Interferon and Neutralizing Antibodies and Thymus Dependence of Interferon and Antibody Production

TATSUO IWASAKI AND TOKUKITI NOZIMA

From the Institute for Virus Research, Kyoto University, Kyoto, Japan

To investigate the defensive roles and production of interferon and antibodies, C3H/He mice were subjected to various immunosuppressive treatments and infected with influenza virus. In infected normal control mice the pattern of pulmonary viral growth can be divided into three phases. The first phase is characterized by an exponential increase of virus titer, the second by a rapid decrease, and the third by a moderate decrease. At the time of transition from the first phase to the second in pulmonary virus growth, interferon could be detected in the tracheobronchial washings of infected mice, but neutralizing antibodies could not.

In infected B cell-deprived mice and infected anti-μ-treated mice, the transition from the first phase to the second occurred without any detectable antibody production, and interferon could be induced in the early stage of infection. However, the pulmonary virus in these mice increased again exponentially until the death of the mice. In infected T cell-deprived mice which could not induce interferon, but produced IgM-neutralizing antibodies, the second phase was not observed after the first phase, but a transient plateau phase could be demonstrated, and then the pulmonary virus increased again exponentially until the death of the mice. In anti-γ-treated infected mice, pulmonary virus growth and production of interferon and neutralizing antibody were almost similar to those of infected normal control mice except for the absence of IgG neutralizing antibody production. Although anti-α-treated infected mice produced interferon and no IgA antibody, the transition from the first exponential increase of pulmonary virus to the second rapid decrease was seen, but then the virus increased exponentially again until the death of the mice.

These results suggest that interferon plays an important role in the transition from the first phase to the second, and that T cells are required for interferon induction in mice infected with influenza virus. These data also suggest that IgA antibodies play an important role in the inhibition of virus propagation in the lungs after the disappearance of interferon. Moreover, infected T cell-deprived mice could produce only IgM neutralizing antibodies, but not IgG and IgA antibodies. Therefore, T cells are required for the production of IgG and IgA antibodies and eventually for defense functions in mice infected primarily with influenza virus.

Submitted for publication September 1, 1976.

The responses of host animals to primary influenza virus infection are complex, and the factors essential for defense against the infection are difficult to define. Possible different roles in the defense mechanisms have been ascribed to interferon and to cell-mediated and humoral immunities, especially respiratory IgA class antiviral antibodies.

Interferon has been detected during both experimental and natural influenza virus infection (1–8). Although considered to be a major factor in host defense against this agent, its role has not been clearly demonstrated. Interferon is also one of the chemical mediators released after immune stimulation and is produced *in vitro* upon exposure of human or mouse leukocytes to general mitogens (9–14), specific antigens (10, 11), anti-lymphocyte sera (15), and allogeneic lymphocytes (16). De Maeyer *et al.* (17) observed the high radiosensitivity of myxovirus-induced circulating interferon and suggested the involvement of lymphocytes in the induction of circulating interferon by these viruses. Although contrasting findings have been reported (18–20), the importance of the spleen in the synthesis of virus-induced interferon has been confirmed by several investigators (21, 22).

Virus-neutralizing antibodies have been detected in the tracheobronchial washings and serum of mice primarily infected with influenza virus (23). Furthermore, in sequential studies of cellular infiltrates from influenza-infected mouse lungs, IgA-bearing lymphocytes exhibited the greatest relative increase (24). At least three immunoglobulin classes have been identified in tracheobronchial secretions and serum, these being differentiated from each other by respective isotypic determinants on the heavy chains of the immunoglobulin molecules. However, little is known about the role of each immunoglobulin class antibody in the defense mechanism of mice primarily infected with influenza virus.

It has been established that a cooperative interaction between thymus-derived (T) and bone marrow-derived (B) lymphocytes is required for the synthesis of antibodies against thymus-dependent antigens. Some antigens such as Type III pneumococcal polysaccharide (S3), bacterial lipopolysaccharides (LPS), polyvinyl-pyrrolidone (PVP), and polymerized flagellin (POL) have been considered to be T-independent (25–28). Immune responses to T-independent antigens are limited in the sense that the antibody produced is solely of the IgM class (29–33). In any case, T cells have been shown to have some regulating functions in the synthesis of each immunoglobulin class of antibody by B cells (34–38), and it has been suggested that IgM antibody production may represent such a limited response that B cells can make it in the absence of T

cell cooperation. However, little is known about the roles of T cells in the production of antibodies in mice infected primarily with influenza virus.

This report describes an attempt to elucidate the roles of interferon and each class of neutralizing antibody in defense mechanisms against primary infection in mice, and to investigate T cell functions in productions of interferon and each class of neutralizing antibody. The data suggest that during the infection, interferon appeared early enough to play a role in the partial suppression of viral propagation in the lungs, and that respiratory secretory IgA neutralizing antibodies play an important role in the inhibition of viral propagation in the lungs after the disappearance of interferon. Moreover, T cells are required for productions of interferon, IgG, and IgA classes of neutralizing antibodies in mice infected with influenza virus.

MATERIALS AND METHODS

Virus. A mouse-adapted strain of AO/PR8 (HONI) virus described previously (39) was employed in all experiments.

Mice. Inbred male mice of the C3H/He strain, 6 to 8 weeks old, were used throughout the experiments. To obtain T cell-deprived (TXXBM)[1] mice, animals were thymectomized at 6 weeks, sublethally irradiated (750 R) 7 to 10 days later, and immediately reconstituted with 2×10^7 syngeneic bone marrow cells. To obtain B cell-deprived (TXXT) mice, thymectomized, sublethally irradiated (TXX) mice were reconstituted with 2 to 5×10^7 syngeneic thymus cells and used for experiments 1 week later.

Thymus and bone marrow cells. Thymuses were removed from 6-week-old C3H/He mice. Single-cell suspensions were obtained by gentle forcing through a stainless steel sieve. After the removal of glass-adherent cells, the suspensions were washed and resuspended in McCoy's 5A modified medium (Difco Laboratories, Detroit, Mich.) supplemented with 10% fetal calf serum (McCoy's 5A-FCS). Bone marrow cells were harvested by ejecting the marrow plug from femoral shafts with cold McCoy's 5A-FCS through a 21-gauge needle, and suspending the cells by repeated gentle aspiration through a 21-gauge needle. After the removal of glass-adherent cells and killing of T cells by treatment with anti-θ (C3H) serum and complement, the suspensions were washed and resuspended in McCoy's 5A-FCS.

Antisera. Monospecific antisera to mouse immunoglobulins were prepared in rabbits. Each immunoglobulin was precipitated by double immunodiffusion in Sepharose against specific antiserum and the resulting precipitin bands were cut out and injected with complete Freund's adjuvant. All antisera were absorbed with light chains isolated from mouse serum IgG2a by means of gel-filtration on Sephadex G-200 in N acetic acid after reduction and alkylation (40). The antibody preparations were monospecific as determined by immunoelectrophoresis and by double diffusion in agarose.

Procedure of anti-immunoglobulin treatment. Mice were treated with 2-mg doses of each anti-immunoglobulin from 4 weeks of age at intervals of 48 hr. Treatment of mice with larger doses of anti-immunoglobulin caused decrement of body weights and increase of mortality of the mice. It was not possible to suppress antibody synthesis by treatment with smaller doses. All mice treated with 2-mg doses were healthy except for the suppression of antibody synthesis. The mice were subjected to inhalation of influenza virus at 7 weeks of age.

Aerosol procedure. Mice were subjected to inhalation of about 40×10^3 pfu of the viruses during a 30-min period according to the method described by Schulman and Kilborne (41).

Pulmonary lesions. The extent of pulmonary lesions was expressed as a percentage of the total lung surface.

Preparations of specimens for assay. At designated intervals 10 mice were bled by dissection of the subaxillary vein, and sera were collected and inactivated at 56°C for 30 min. Then 0.1 ml of phosphate buffered saline was injected into the tracheobronchus and aspirated into a syringe. The pooled washings were centrifuged at 1500 rpm for 5 min, then inactivated at 56°C for 30 min. Both specimens were stored at 4°C until assay. Lungs from 10 mice were removed aseptically and pooled, and then ground in a glass tube with Teflon grinders to make a 10% suspension v/v in phosphate-buffered saline. These suspensions were centrifuged 7500 rpm for 15 min. Supernatant fluids were stored at −70°C until assay.

Removal of IgM, IgG, and IgA by precipitation with specific antisera. Mixtures of 0.8 ml of the specimens (tracheobronchial washings and sera) to be absorbed and 0.1 ml of each appropriate antiserum of anti-γ and anti-α (for estimation of IgM antibodies), or anti-μ and anti-α (for estimation of IgG antibodies), or anti-μ and anti-γ (for estimation of IgA antibodies) were incubated in a water bath at 37°C for 30 min, then kept at 4°C overnight. After centrifugation, the supernatants were examined for virus neutralizing activity of IgM, IgG, or IgA.

Virus titrations. Chick embryo fibroblast monolayers were washed with prewarmed (36°C) phosphate-buffered saline and inoculated with 0.2 ml of the appropriate dilution of the virus suspension per bottle, using four bottles for each suspension. After adsorption at 36°C for 90 min, the virus suspension was removed, and monolayers were washed with phosphate-buffered saline, then 4 ml of the first overlay medium were added. After 3 days of incubation at 36°C, 2 ml of the second overlay medium were added, and plaques were counted at the 4th day. The first overlay medium consists of medium-199 supplemented with 2% skim milk, 0.025% pancreatin, 0.9% special agar-Noble and 0.01% DEAE-dextran. The second overlay medium consists of medium-199 supplemented with 0.01% neutral red and 0.9% special agar-Noble.

Assay of interferon. Appropriate dilutions of tracheobronchial washings and sera were incubated for 16 hr on L cell monolayer cultures. After the removal of the fluids, the cell monolayers were washed with phosphate-buffered saline, and 200 pfu of vesicular stomatitis virus were inoculated into each cell culture. Adsorption of the virus was allowed to proceed for 60 min at 37°C. An overlay medium consisting of Eagle's minimum essential medium supplemented with 2% FCS was added to each cell culture. The cultures were incubated for 48 hr and the number of plaques was determined. Interferon titers are expressed in units, one unit corresponding to the reciprocal of the dilution which reduced the number of plaques by 50%.

Assay of neutralizing antibody. Neutralizing antibody titers of specimens were determined by the 50% plaque reduction method. Mixtures of equal volumes of virus suspension containing approximately 200 pfu of AO/PR8 virus and of appropriate dilutions of the specimens were incubated at 37°C for 40 min. Thereafter, the infectivity of the residual virus was

[1] Abbreviations used in this paper: TXXBM, T cell deprived; TXXT, B cell deprived; TXX, sublethally irradiated.

assayed by the plaque technique mentioned above. Neutralizing antibody titers of specimens were expressed in units, one unit corresponding to the reciprocal of the dilution which reduced the number of plaques by 50%.

RESULTS

The course of infection and the production of interferon and neutralizing antibodies in normal control mice were compared to TXX, TXXT, TXXBM, anti-μ-treated, anti-γ-treated and anti-α-treated mice.

Pulmonary virus propagation and production of interferon and neutralizing antibodies in infected normal control mice. As shown in Figure 1, the titer of pulmonary virus increased exponentially until the 4th day after infection, then decreased first rapidly, then gradually. Therefore, in the lungs of mice sublethally infected with influenza virus the pattern of viral growth can be divided into three phases. The first phase is characterized by an exponential increase of virus titer, the second by a rapid decrease, and the third by a moderate decrease. The lung lesions appeared on the 4th day and developed until the 7th day after infection. The titer of interferon increased to reach a maximum on the 5th day after infection in the tracheobronchial washings and on the 7th day in the serum, and then decreased rapidly.

In the tracheobronchial washings IgM neutralizing antibodies could be detected first on the 5th day after infection, and their titers reached a peak on the 7th day, then decreased gradually. IgA antibodies were initially detected as early as the 7th day after infection, and subsequently their titer increased until 12th to 14th day, and then decreased gradually. IgG antibodies were detected first on the 9th day, and their peak titer was reached on the 12th day after infection. IgA-neutralizing antibodies were predominant in each immunoglobulin class of antibody in the tracheobronchi of the infected mice. In the serum IgM antibodies appeared on the 5th day, and their titer reached a peak on the 7th day and then decreased gradually. A more protracted formation of IgG antibody followed, and IgA antibody appeared late. These results are shown in Figure 2.

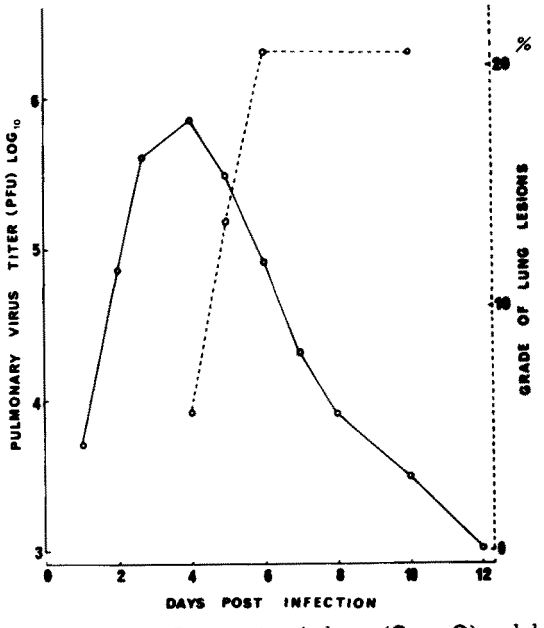

Figure 1. Growth of influenza virus in lungs (O——O) and development of lung lesions (O-----O) of normal control mice sublethally infected with influenza virus.

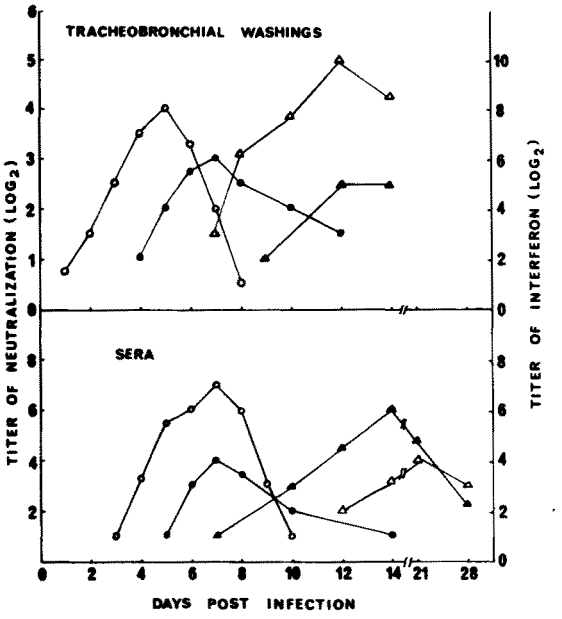

Figure 2. Production of interferon and neutralizing antibodies of each immunoglobulin class in normal control mice infected primarily with a sublethal dose of influenza virus. Interferon (O), IgM (●), IgG (▲), IgA (△).

Any class of neutralizing antibodies in the tracheobronchus and serum were not detectable until the 5th day after infection. It is, therefore, reasonable to consider that the major factor responsible for the transition from the first phase to the second in pulmonary virus growth is interferon rather than antiviral antibody.

Virus growth and absence of production of interferon and neutralizing antibodies in infected TXX mice. As shown in Figure 3, pulmonary virus increased exponentially until the death of the mice on the 5th day after infection. The lung lesions developed over the whole lung surface and their progress paralleled the extent of pulmonary virus growth. No significant increase of interferon or neutralizing antibody was observed in either the tracheobronchus or the serum. The marked differences in pulmonary virus titers between TXX and normal control mice were observed as early as 2 days after infection. The 100-fold elevations observed in titer of TXX mice compared to that of normal control mice may be attributable to the absence of interferon, although some other early effects of thymectomy and irradiation on host resistance can not be excluded.

Effects of T cells on growth of pulmonary virus, development of lung lesions and interferon production. In a study of the effects of T cells on growth of pulmonary virus, development of lung lesions and interferon production, TXXT mice were infected. Figures 4 and 5 show the strong T cell dependence of interferon production by influenza virus. The titer of the pulmonary virus increased exponentially until the 4th day, and then decreased rapidly until the 6th day after infection. However, with the decrease of interferon production, the titer of pulmonary virus increased again exponentially until the death of the mice. The extent of the lung lesions paralleled that of pulmonary virus growth. Neutralizing antibodies were not detectable in the tracheobronchus or serum throughout the life of the mice.

Effects of B cells on growth of pulmonary virus, development of lung lesions and production of interferon and neutralizing antibodies. The experimental results, as shown in Figure 4,

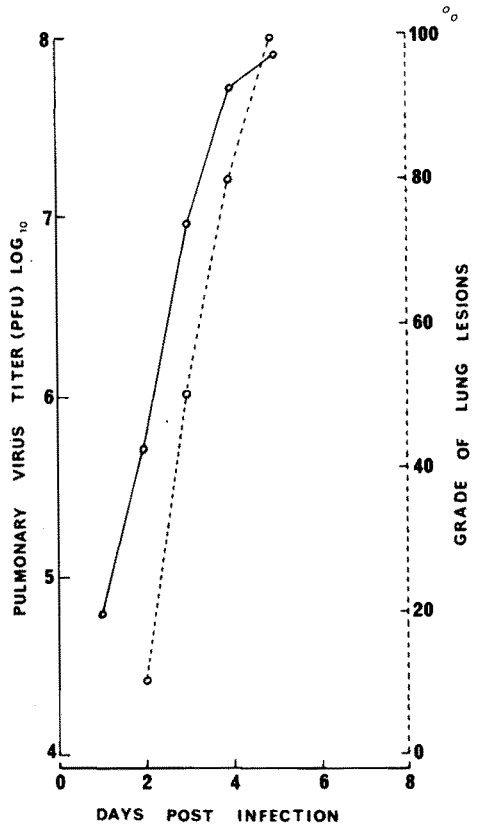

Figure 3. Growth of influenza virus in lungs (O——O) and development of lung lesions (O-----O) of infected TXX mice.

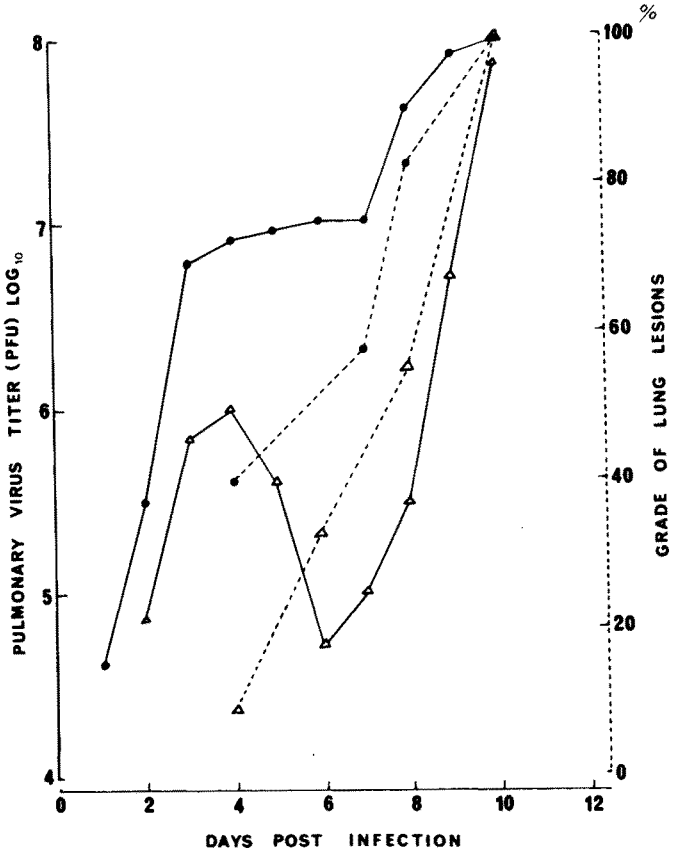

Figure 4. Virus propagation in lungs (——) and development of lung lesions (-----) in influenza-infected TXXT (△) and TXXBM (●) mice.

demonstrate a characteristic pattern of pulmonary virus growth: 1) an exponential increase of pulmonary virus titer as well as in infected normal control mice, 2) a transient plateau phase, instead of the rapid decrease phase observed in infected normal control mice, and 3) an exponential increase phase until the death of the mice. The development of lung lesions paralleled the extent of pulmonary virus growth. No significant increase of interferon was observed in the tracheobronchus or serum, but neutralizing antibodies were detectable, although in low titers. The second plateau phase may be due to the appearance of neutralizing antibodies. These antibodies were completely absorbed by rabbit anti-mouse μ serum, but not by anti-mouse γ or α serum, as shown in Figure 6. Therefore, IgM class of neutralizing antibody was found to be solely produced in infected TXXBM mice, but IgG or IgA class of neutralizing antibody was not.

Virus propagation, development of lung lesions and production of interferon in infected mice pretreated with anti-μ-antibodies. Antigen receptors located on the surface of B cells are considered to be representatives of the eventual cell products with regard to both specificity and immunoglobulin class. Therefore, antibodies against a particular class of heavy chain determinants displayed by immunoglobulin receptors have been used by several investigators for selective inhibition of the production of corresponding immunoglobulin classes of antibody in mice *in vivo* (42–46). Next experiments were designed to investigate virus propagation in the lungs and the development of lung lesions in infected mice pretreated with anti-μ antibodies. Pulmonary virus increased exponentially until the 4th day and then decreased rapidly until the 6th day after infection, but increased again exponentially until the death of the mice, as shown in Figure 7. The mice could normally produce interferon, but could produce no IgM antibodies (Fig. 8). These results substantiate our previous proposition that transition from the first phase of exponential increase of virus propagation in the lungs to the second phase of decrease may be due to interferon, but not to antibodies. It is

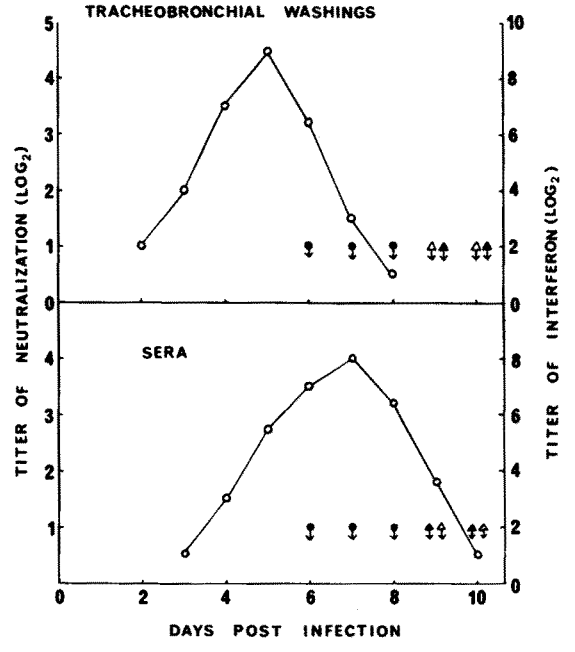

Figure 5. Production of interferon and neutralizing antibodies of each immunoglobulin class in TXXT mice infected with influenza virus. Interferon (O), IgM (●), IgG (▲), IgA (△).

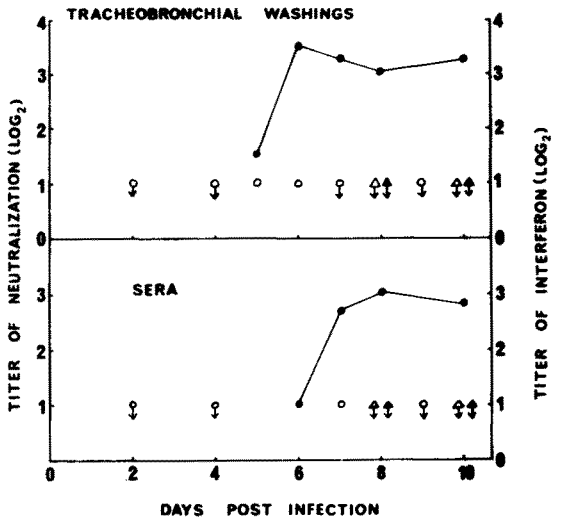

Figure 6. Production of interferon and neutralizing antibodies of each immunoglobulin class in TXXBM mice infected with influenza virus. Interferon (○), IgM (●), IgG (▲), IgA (△).

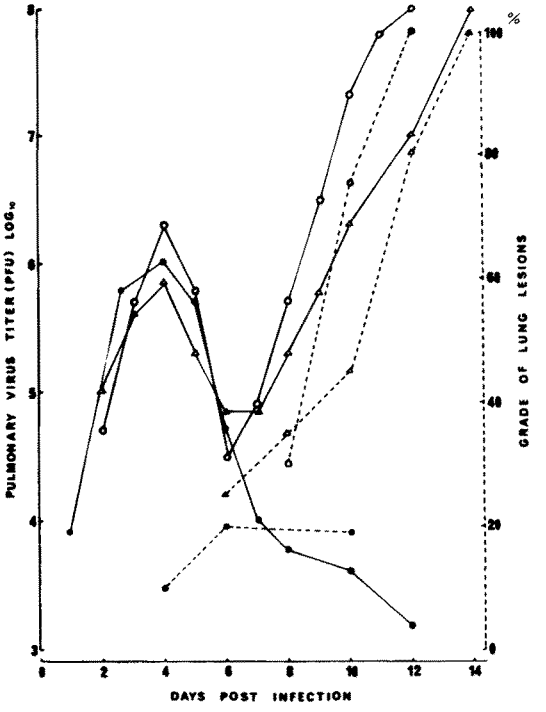

Figure 7. Virus propagation in lungs and development of lung lesions of anti-μ-treated (○), anti-γ-treated (●) and anti-α-treated (△) mice infected with influenza virus.

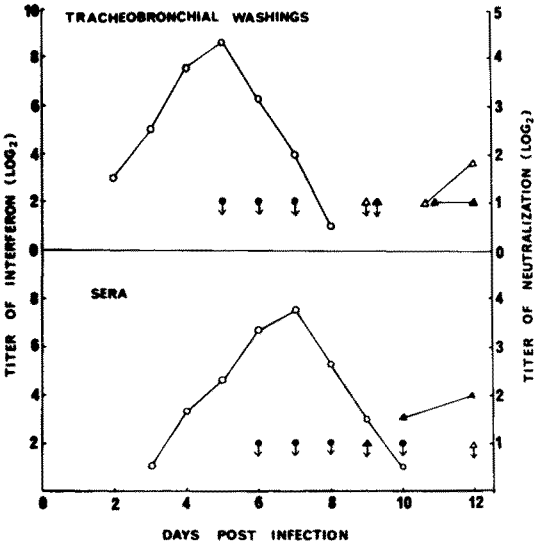

Figure 8. Production of interferon and neutralizing antibodies of each immunoglobulin class in anti-μ-treated mice infected with influenza virus. Interferon (○), IgM (●), IgG (▲), IgA (△).

also suggested that suppression of antibody production preceded by decrease of interferon production may lead to the third phase of exponential increase of pulmonary viral titer in infected mice treated with anti-μ antibodies.

Effect of pretreatment with anti-γ antibodies on virus growth in lungs, development of lung lesions, production of interferon and antibodies in infected mice. The results show (Fig. 7) that the patterns of pulmonary virus growth and development of lung lesions in the infected mice pretreated with anti-γ antibodies were almost the same as those in the infected normal control mice. As shown in Figure 9, selective suppression of IgG antibody production was observed in both tracheobronchial washings and serum. But interferon could be produced normally. It is, therefore, likely that IgG antibodies do not play an important role in the defense mechanisms of primarily infected mice.

Effect of pretreatment with anti-α antibodies on pulmonary virus growth, development of lung lesions and production of interferon and antibodies in infected mice. As shown in Figure 7, the patterns of pulmonary virus growth and development of lung lesions in the infected mice pretreated with anti-α antibodies were almost the same as those in infected TXXT mice and the infected mice pretreated with anti-μ antibodies. The results also show (Fig. 10) a normal production of interferon and antibodies except for a selective suppression of IgA antibody production. In the infected mice pretreated with anti-α antibodies, the second gradual increase of pulmonary virus titer occurred in spite of normal production of interferon, IgM, and IgG antibodies. It is, therefore, reasonable to consider that IgA antibodies play an important role in inhibiting the second increase of pulmonary virus titer after the disappearance of interferon.

DISCUSSION

On the basis of our experimental results, it seems reasonable to propose that influenza viral growth in the lungs of sublethally infected normal control mice can be divided into three phases: first an exponential increase of virus titer, second a rapid decrease, and third a gradual decrease. For an understanding of defense mechanisms against influenza infection, it is necessary to determine the factors involved in the inhibition of viral multiplication at each of these stages.

From our experimental results, the transition from the first to the second phase of viral growth seems to be caused by the action of virus-induced interferon, since this pattern of viral behavior was also observed in infected TXXT, and anti-μ-treated mice without any significant antibody production. However, the possible role of other cell-mediated immune responses has not been ruled out, although studies with bursectomy and thymectomy in an avian model suggested that cell-mediated immunity is less important than humoral immunity in recovery from influenza virus infection (47). In our

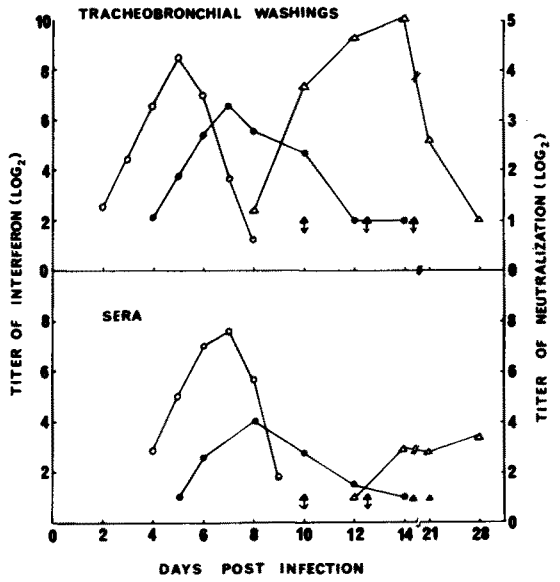

Figure 9. Production of interferon and neutralizing antibodies of each immunoglobulin class in anti-γ-treated mice infected with influenza virus. Interferon (O), IgM (●), IgG (▲), IgA (△).

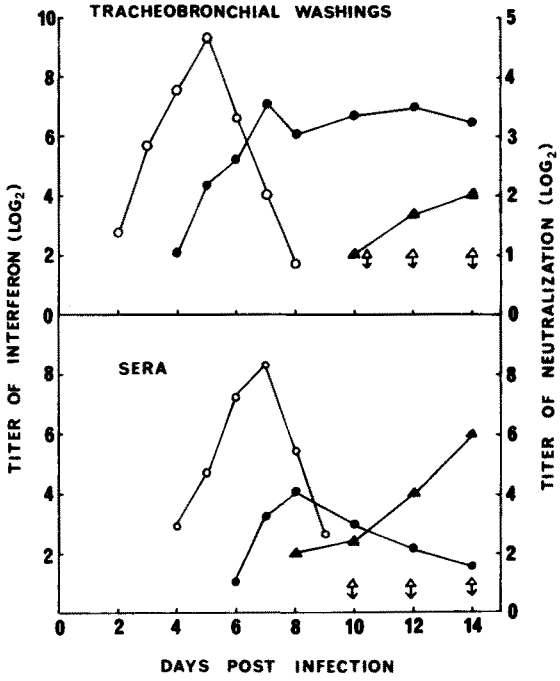

Figure 10. Production of interferon and neutralizing antibodies of each immunoglobulin class in anti-α-treated mice infected with influenza virus. Interferon (O), IgM (●), IgG (▲), IgA (△).

experiments on interferon induction *in vivo*, a striking T cell dependency was demonstrated. In the T cell-mediated interferon induction *in vivo*, it remained unsolved whether the T cell itself was an interferon-producing cell. Recently, Tsukui *et al.* (submitted to the publication) showed that the main interferon-producing cells in spleen cell cultures from normal mice upon challenge with influenza virus were T cells, and that the presence of macrophages augmented the interferon production by T cells in a more efficient manner than the absence of macrophages, such as in vaccinia and herpes infections (48–51). However, non-T cells, especially macrophages, were not incapable of interferon production *in vitro*. In fact, various kinds of cells may potentially become interferon-producing cells *in vitro*, but in some cases, interferon production *in vivo* has been shown to be clearly dependent on the presence of functional lymphocytes by the experimental findings on x-ray irradiated, anti-lymphocyte serum treated, or certain immunosuppressive hormone-treated mice (17, 21, 22, 52–54). In our experimental case, it can be assumed that main interferon-producing cells are T cells, or under T cell control, and that producibility of interferon of other kinds of cells are suppressed so strongly by unknown factor(s) that we can not detect its activity.

A refractory state of interferon production in the late stages of infection may be ascribed to destruction of virus receptors or dysfunction of T cells when it just as well could relate to diminution in virus concommitant with the local immune control of the infections process, although on metabolically active cells the extremely rapid regeneration of sialic acid receptors would make this assumption implausible.

An alternative explanation for a refractory state of interferon production is that interferon itself mediates hyporeactivity, since production of interferon is frequently depressed when cell cultures are preincubated with large doses of interferon (55). A somewhat different explanation is that the postulated protein induced by interferon mediates a refractory state (56–58). Studies along this line in our system are now in progress.

The results presented here further demonstrate that the infected normal control mice began to produce neutralizing antibodies of the IgM class after interferon production began to decrease. In the infected TXXBM mice, viral growth was found to be incapable of transition from the first phase to the second in the presence of IgM antibodies alone without interferon production, although a slight inhibition of virus multiplication was detected. So it is possible that IgM antibodies play a slightly inhibitory role in virus propagation after the disappearance of interferon in infected normal control mice.

We have also shown that infected TXXBM mice produced neutralizing antibodies of the IgM class, but neither IgG-nor IgA-neutralizing antibodies. We confirmed that reconstitution with T cells restored the ability to produce IgG and IgA antibodies (unpublished observation), but IgM antibody production was found to be T cell independent. Virelizier *et al.* recently reported (59) that thymus-deprived mice did not produce antibody against influenza virus hemagglutinin, and that the inability of thymus-deprived mice to produce antibody against influenza virus hemagglutinin could be overcome by repeating the injection of antigen. In view of these facts, it seems likely that IgM antibody can be produced by successive stimulation with increasing amounts of virus grown progressively in the lungs of infected TXXBM mice. Furthermore, our virus neutralization method is more sensitive than single-radial-immunodiffusion method used by Virelizier *et al.*

Burns and Allison (60) have observed that infection with Sindbis virus in nude mice resulted in high titers of neutralizing antibody, solely of the IgM class; this titer was comparable to that in control littermates and was sustained for 2 weeks. We also observed that nude mice infected with influenza virus produced only IgM-neutralizing antibody (unpublished data).

No difference was found in the extent and pattern of virus propagation between anti-γ-administered infected mice and infected normal control mice. Thus, it is likely that IgG antibodies do not play an important role in defense against influenza virus infection. Although it remains unexplained why

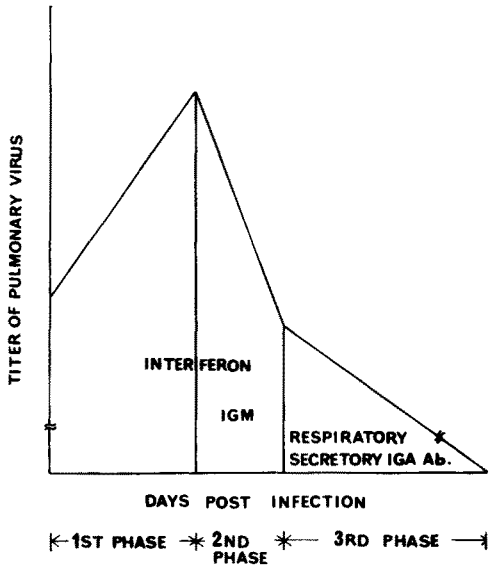

Figure 11. Major defensive factors at each stage of pulmonary virus growth in mice infected primarily with a sublethal dose of influenza virus.

IgM or IgG antibodies alone cannot convert the second phase to the third, we are forced to consider that under experimental circumstances such as virus-infected tracheobronchi in mice, the production of IgM or IgG is not allowed to proceed at a level high enough to suppress viral multiplication.

In anti-α-administered infected mice, viral growth in the lungs could not make the transition from the second phase to the third. Thus, we emphasize that IgA antibodies can play a dominant role in the inhibition of viral multiplication in the third phase.

As described previously (39), infected mice pretreated with lymphocytosis-promoting factor obtained from *Bordetella pertussis* were shown to be capable of suppressing the production of circulating antibody against influenza virus without affecting the ability to produce respiratory secretory antibody. It is significant that no difference could be observed in the extent and pattern of virus propagation between the infected control mice and infected mice pretreated with lymphocytosis-promoting factor. Taken together, these findings indicate that respiratory secretory antibodies play a major defensive role in the third phase in mice infected with influenza virus infection, especially in view of the virus-inhibitory activity of secretory IgA which is the dominant immunoglobulin in respiratory secretion and which appears in the third phase. In this respect, it is tempting to propose separate roles of interferon and of the antibodies of each immunoglobulin class, as shown in Figure 11.

REFERENCES

1. Isaacs, A. 1963. Interferon. *In* Advances in Virus Research. Vol. 10. Edited by K. M. Smith and M. A. Lauffer. Academic Press, New York. P. 1.
2. Baron, S. 1963. Mechanism of recovery from viral infection. *In* Advances in Virus Research. Vol. 10. Edited by K. M. Smith and M. A. Lauffer. Academic Press, New York. P. 39.
3. Wheelock, E. F., and W. A. Sibley. 1964. Interferon in human serum during clinical viral infections. Lancet 2:382.
4. Gresser, I., and H. B. Dull. 1964. A virus inhibitor in pharyngeal washings from patients with influenza. Proc. Soc. Exp. Biol. Med. 115:192.
5. Wheelock, E. F., and W. A. Sibley. 1965. Circulating virus, interferon and antibody after vaccination with the 17-D strain of yellow-fever virus. N. Engl. J. Med. 273:194.
6. Petrali, J. K., T. C. Merigan, and J. R. Wilbur. 1965. Circulating interferon after measles vaccination. N. Engl. J. Med. 273:198.
7. Jao, R. L., E. F. Wheelock, and G. G. Jackson. 1965. Interferon study in volunteers infected with Asian influenza. J. Clin. Invest. 44:1062.
8. Cate, T. R., R. G. Douglas, Jr., and R. B. Couch. 1969. Interferon and resistance to upper respiratory virus illness. Proc. Soc. Exp. Biol. Med. 131:631.
9. Wheelock, E. F. 1965. Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin. Science 149:310.
10. Friedman, R. M., and H. L. Cooper. 1967. Stimulation of interferon production in human lymphocytes by mitogens. Proc. Soc. Exp. Biol. Med. 125:901.
11. Wallen, W. C., J. H. Dean, and D. O. Lucas. 1973. Interferon and the cellular immune response: Separation of interferon-producing cells from DNA-synthetic cells. Cell. Immunol. 6:110.
12. Epstein, L. B., H. W. Kreth, and L. A. Herzenberg. 1974. Fluorescence-Activated cell sorting of human T and B Lymphocytes. II. Identification of the cell type responsible for interferon production and cell proliferation in response to mitogens. Cell. Immunol. 12:407.
13. Epstein, L. B., M. J. Cline, and T. C. Merigan. 1970. *In* Proceedings of the 5th Leukocyte Culture Conference. Edited by J. E. Harris. Academic Press, New York. P. 506.
14. Epstein, L. B., M. J. Cline, and T. C. Merigan. 1971. The interaction of human macrophages and lymphocytes in the phytohemagglutinin-stimulated production of interferon. J. Clin. Invest. 50:744.
15. Falcoff, R., R. Oriol, and S. Iscaki. 1972. Lymphocyte stimulation and interferon induction by 7S anti-human lymphocyte globulins and their uni- and divalent fragments. Eur. J. Immunol. 2:476.
16. Gifford, G. E., A. Tibor, and D. L. Peavy. 1971. Interferon production in mixed lymphocyte cell cultures. Infect. Immun. 3:164.
17. De Maeyer, E., J. De Maeyer-Guignard, and P. Jullien. 1969. Interferon synthesis in x-irradiated animals. III. The high radiosensitivity of myxovirus-induced circulating interferon production. Proc. Soc. Exp. Biol. Med. 131:36.
18. De Somer, P., and A. Bilian. 1966. Interferon production by the spleen of rats after intravenous injection of Sindbis virus or heat-killed *Escherichia coli*. Arch. Gesamte Virusforsch. 19:143.
19. Borecký, L., and V. Lackovič. 1967. The cellular background of interferon production *in vivo*. Comparison of interferon induction by Newcastle disease virus and *Bordetella pertussis*. Acta Virol. 11:150.
20. Hellmann, W., and H. Kohlkage. 1973. Effects of splenectomy on production of virus induced interferon in rabbits. Nature (New Biol.) 241:239.
21. Subrahmanyan, T., and C. Mims. 1966. Fate of intravenously administered interferon and the distribution of interferon during virus infections in mice. Br. J. Exp. Pathol. 47:168.
22. Van Rossum, W., and P. De Somer. 1966. Some aspects of the interferon production *in vivo*. Life Sci. 5:105.
23. Fazekas De St. Groth, S., and M. Donneley. 1950. Studies in experimental immunology of influenza. 3. The antibody response. Aust. J. Exp. Biol. Med. Sci. 28:61.
24. Scott, G. H., and J. S. Walker. 1976. Immunoglobulin-bearing cells in lungs of mice infected with influenza virus. Infect. Immun. 13:1525.
25. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells on B cell responses to antigen. *In* Advances in Immunology. Vol. 15. Edited by F. J. Dixon and H. G. Kunkel. Academic Press, New York. P. 1.
26. Howard, J. G., G. H. Christie, B. M. Courtenay, E. Leuchars, and A. J. S. Davies. 1971. Studies on immunological paralysis. VI. Thymic-independence of tolerance and immunity to type III pneumococcal polysaccharide. Cell. Immunol. 2:614.

27. Andersson, B., and H. Blomgren. 1971. Evidence for thymus-independent humoral antibody production in mice against polyvinyl pyrrolidone and *E. coli* lipopolysaccharide. Cell. Immunol. 2:411.
28. Armstrong, W. D., E. Diener, and G. R. Shellam. 1969. Antigen-reactive cells in normal, immunized, and tolerant mice. J. Exp. Med. 129:393.
29. Baker, P. J., and P. W. Stashak. 1969. Quantitative and qualitative studies on the primary antibody response to pneumococcal polysaccharides at the cellular level. J. Immunol. 103:1342.
30. Braley, H. C., and M. J. Freeman. 1971. Strain differences in the antibody plaque-forming cell responses of inbred mice to pneumococcal polysaccharide. Cell. Immunol. 2:73.
31. Britton, S., and G. Möller. 1968. Regulation of antibody synthesis against *Escherichia coli* endotoxin. I. Suppressive effect of endogenously produced and passively transferred antibodies. J. Immunol. 100:1326.
32. Barthold, D. R., B. Prescott, P. W. Stashak, D. F. Amsbaugh, and P. J. Baker. 1974. Regulation of the antibody response to type III pneumococcal polysaccharide. III. Role of regulatory T cells in the development of an IgG and IGA antibody response. J. Immunol. 112:1042.
33. Melchers, F., and J. Anderson. 1974. *In* Cellular Selection and Regulation in the Immune Response. Edited by G. M. Edelman. Raven Press, New York. P. 217.
34. Cheers, C., and J. F. A. P. Miller. 1972. Cell-to-cell interaction in the immune response. IX. Regulation of hapten-specific antibody class by carrier priming. J. Exp. Med. 136:1661.
35. Grumet, F. C. 1972. Genetic control of the immune response. A selective defect in immunologic (IgG) memory in nonresponder mice. J. Exp. Med. 135:110.
36. Mitchell, F. F., F. C. Grumet, and H. O. McDevitt. 1972. Genetic control of the immune response. The effect of thymectomy on the primary and secondary antibody response of mice to poly-L(Tyr, Glu)-poly-D, L-Ala-poly-L-Lys. J. Exp. Med. 135:126.
37. Ordal, J. C., and F. C. Grumet. 1972. Genetic control of the immune response. The effect of graft-versus-host reaction on the antibody response to poly-L(Tyr, Glu)-poly-D, L-Ala-poly-L-Lys in nonresponder mice. J. Exp. Med. 136:1195.
38. Braley-Mullen, H. 1974. Regulatory role of T cells in IgG antibody formation and immune memory to type III pneumococcal polysaccharide. J. Immunol. 113:1909.
39. Iwasaki, T., and T. Nozima. 1970. Effect of lymphocytosis-promoting factor (LPF) on the appearance of antibodies in mice infected with influenza virus. J. Immunol. 104:1293.
40. Fleischman, J. B., R. Pain, and R. R. Porter. 1961. Arch. Biochem. Biophys. Suppl. 1: 174.
41. Schulman, J. L., and E. D. Kilbourne. 1963. Experimental transmission of influenza virus infection in mice. 1. The period of transmissibility. J. Exp. Med. 118:257.
42. Manning, D. D., and J. W. Jutila. 1972. Immunosuppression of mice injected with heterologous anti-immunoglobulin heavy chain antisera. J. Exp. Med. 135:1316.
43. Manning, D. D., and J. W. Jutila. 1972. Immunosuppression in mice injected with heterologous anti-immunoglobulin antisera. J. Immunol. 108:282.
44. Lawton, A. R., R. M. Asofsky, M. D. Hylton, and M. D. Cooper. 1972. Suppression of immunoglobulin synthesis in mice. I. Effects of treatment with antibody to μ-chain. J. Exp. Med. 135:277.
45. Murgita, R. A., C. A. Mattioli, and T. B. Tomasi, Jr. 1973. Production of a runting syndrome and selective γ A deficiency in mice by the administration of anti-heavy chain antisera. J. Exp. Med. 138:209.
46. Lawton, A. R., R. M. Asofsky, J. M. Davie, and M. B. Hylton. 1973. Suppression of immunoglobulin synthesis in mice: Age dependence of anti-μ suppression and effects of anti-(γ_1 + γ_2), anti-γ_2, and anti-α. Fed. Proc. 32:1012.
47. Portonoy, J., K. Bloom, and T. C. Merigan. 1969. Induction of delayed hypersensitivity to influenza and mumps viruses in mice. J. Immunol. 103:844.
48. Epstein, L. B., D. A. Stevens, and T. C. Merigan. 1972. Selective increase in lymphocyte interferon response to vaccinia antigen after revaccination. Pro. Natl. Acad. Sci. 69:2632.
49. Merigan, T. C., D. A. Stevens, L. E. Rasmussen, and L. B. Epstein. 1973. *In* A reexamiantion of non-specific resistance to infection. Edited by W. Braun and J. Ungar. Karger, Basel. P. 430.
50. Rasmussen, L. E., G. W. Jordan, D. A. Stevens, and T. C. Merigan. 1974. Lymphocyte interferon production and transformation after herpes simplex infections in human. J. Immunol. 112:728.
51. Valle, M. J., A. M. Bobrove, S. Strober, and T. C. Merigan. 1975. Immune specific production of interferon by human T cells in combined macrophage-lymphocyte cultures in response to herpes simplex antigen. J. Immunol. 114:435.
52. Barth. R. F., R. M. Friedman, and R. A. Malmgren. 1969. Depression of interferon production in mice after treatment with anti-Lymphocyte serum. Lancet 2:723.
53. De Maeyer-Guignard, J., and E. De Maeyer. 1971. Effect of antilymphocytic serum on circulating interferon in mice as a function of the inducer. Nature (New Biol.) 229:212.
54. Postic, B., C. De Angelis, M. K. Breining, and M. Ho. 1967. Effects of cortisol and adrenalectomy on induction of interferon by endotoxin. Proc. Soc. Exp. Biol. 125:89.
55. Finter, N. B. 1973. Interferon and interferon inducers. New York, Elsevier. P. 598.
56. Chany, C., and M. Vignal. 1970. Effect of prolonged interferon treatment on mouse embryonic fibroblasts transformed by murine sarcoma virus. J. Gen. Virol. 7:203.
57. Rousset, S. 1974. Refractory state of cells on interferon induction. J. Gen. Virol. 22:9.
58. Borden, E. C., and F. A. Murphy. 1971. The interferon Refractory state: *In vivo* and *in vitro* studies of its mechanism. J. Immunol. 106:134.
59. Virelizier, J. L., R. Postlethwaite, G. C. Schild, and A. C. Allison. 1974. Antibody responses to antigenic determinants of influenza virus hemagglutinin. J. Thymus dependence of antibody formation and thymus independence of immunological memory. J. Exp. Med. 140:1559.
60. Burns, W. H., and A. C. Allison. 1975. Virus infections and the immune responses. *In* The Antigens. Vol. 3. Edited by M. Sela. Academic Press, New York. P. 479.