INFECTION AND IMMUNITY, June 1974, p. 991-998

Vol. 9, No. 6
Printed in U.S.A.

Specific and Nonspecific Cell-Mediated Resistance to Influenza Virus in Mice

MEHDI SHAYEGANI,[1] FLORENCE S. LIEF, AND STUART MUDD

Schools of Medicine and Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174

Received for publication 18 December 1973

We found that influenza virus had the capacity to replicate in the peritoneal macrophages of normal mice, as revealed by the development of hemadsorption and the appearance intracellularly of S and V antigens. Cell-mediated resistance was studied in mice infected with influenza virus or a bacterial sytem of induction-elicitation. In the homologous system, mice were injected intraperitoneally or exposed by aerosol to a sublethal dose of an egg-adapted swine strain of influenza virus. In the heterologous system, they were infected repeatedly with *Staphylococcus aureus* and elicited by subcutaneous or aerosol administration of staphylococcal antigens. The peritoneal macrophages from mice specifically or nonspecifically immunized were significantly more resistant than those from normal mice. Also longer survival to in vivo challenge by the mouse-adapted virus, as compared with normal mice, was indicated in bacterially stimulated mice.

Definition: induction and elicitation. By induction, in the present context, is meant the evocation of delayed-type hypersensitivity in response to appropriate antigens, typically of a living pathogenic agent. In the present communication, induction is by repeated infection with living *Staphylococcus aureus*.

By elicitation is meant the evocation of lymphokines and the activation of macrophages in response to antigens homologous with the pathogenic agent. In this communication, elicitation is by staphylococcal phage lysate (SPL). More commonly, BCG is used as inducer and BCG, OT, or purified protein derivative as eliciting agent.

Specific induction and stimulation of laboratory animals by certain microorganisms result in activation of macrophages and increased capability of intracellular destruction of unrelated as well as related microorganisms.

With staphylococcal infection in mice, activation of peritoneal cells and significant increases in intracellular destruction by these cells of *S. aureus* in vitro were demonstrated in mice infected repeatedly with *S. aureus* and elicited by staphylococcal antigens (16). Such treated mice also were shown to be protected against challenge with vaccinia virus in vivo (1).

Although the important role of activated macrophages in bacterial infections has been extensively investigated, the defensive role of these cells in viral infections is less well known. Relationships between viruses and leukocytes have been reviewed by Gresser and Lang (5) without consideration of cell-mediated immunity. More recently, Glasgow (4) surveyed the significance of cellular immunity in host resistance to viral infections. The cellular immune function with respect to influenza virus infections has received only limited attention. Feinstone et al. (3) injected an influenza virus vaccine in complete Freund adjuvant into the footpads of mice and used splenic lymphocytes and inhibition of macrophage migration as their test system. Waldman et al. (19) evaluated cellular and humoral immunity in the guinea pig after systemic or local administration of high and low doses of a killed influenza virus vaccine. Also using inhibition of macrophage migration as their indicator system, they examined cells from bronchial washings as well as splenic lymphocytes.

In the present study, it was found that influenza virus had the capacity to replicate in peritoneal macrophages from normal mice, as revealed by the development of hemadsorption and the formation of S (soluble or ribonucleoprotein) and V (viral or capsid) antigens in the infected cells. Thus, a new in vitro system has become available whereby the production of resistance to influenza virus at the cellular level can be measured. Therefore, as our test system, we employed the reaction to virus challenge of peritoneal cells derived from virus immunized

[1] Present address: Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. 12201.

and from bacterially induced and elicited mice as compared to the reactions of cells from normal animals.

MATERIALS AND METHODS

Source of mice. Female albino Swiss random-bred mice (Huntington Farms, Inc., West Conshohocken, Pa.) weighing 18 to 22 g were used throughout this study.

Influenza virus. A laboratory strain of A/Swine/Iowa 15/31 maintained in eggs was adapted to mice by seven serial lung to lung transfers. The mouse-grown and -adapted virus was passed once in 10- to 11-day-old embryonated chicken eggs. The resulting infected allantoic fluids were pooled and distributed in ampoules and frozen at −80 C until used. Before each experiment, the virus egg infectivity titers of these pools were assayed in the usual manner (6). Its mouse infectivity titers were measured by exposing in an aerosol chamber (see below) a group of six mice each to falling 10-fold dilutions of virus in normal allantoic fluid. The exposed mice, as well as a control group which was exposed to aerosolized normal allantoic fluid, were observed for a period of 12 days. At the end of that time, all survivors were sacrificed. Any animals that died, as well as the killed animals, were examined for the extent of their lung lesions, which were scored 1 through 4 plus. The egg infectivity (EID_{50}) and mouse infectivity and minimal lethal titers (LD_{50}) were computed according to the Reed-Muench formula (14).

Staphylococcal strain. *S. aureus* strain 18Z described previously (8) was used for infection of mice. Bacterial suspensions were made from 18-h cultures grown in Trypticase soy broth (TSB; Baltimore Biological Laboratories, Baltimore, Md.) and washed twice with 0.85% NaCl containing 1% TSB.

Aerosol chamber. The aerosol chamber had been devised for use in earlier influenza virus research (F. S. Lief and L. Syrucek, unpublished data). It consisted of an air-tight stainless-steel chamber 12 inches (about 30 cm) wide, 19 inches (about 47.5 cm) deep, and 16 inches (about 40 cm) high with a clear 0.5 inch (about 1.25 cm)-thick Plexiglass removable front. The mice were placed in the chamber in shallow wire mesh containers which could be stacked with air spaces in between. At the inlet to the chamber was a glass Vaponephrine nebulizer (Vaponephrine Co., Portland, Ore.) connected to a tank of air under pressure. The flow of air through the nebulizer was controlled by a flow meter. At the terminal end, the air passing through the chamber was sterilized by passage through Wescodyne (West Chemical Products, Inc., New York, N.Y.). In practice, it was found that nebulization of 2 ml of a virus suspension gave highly reproducible results. With a flow of 3 liters of air per min, it required 20 min. At the end of that time, the nebulizer was bypassed and the chamber was flushed with air alone for at least 10 min, after which the chamber was opened and the mice were removed.

Immunization and challenge of mice with influenza virus. For immunization, mice were either inoculated intraperitoneally with 0.5 ml of the original egg-adapted virus which contained $10^{8.7}$ EID_{50} or were exposed by aerosol to a sublethal dose of the same virus. For in vivo challenge, mice were exposed to 10 to 100 LD_{50} of mouse-adapted virus by aerosol or a dose that would kill six out of six animals (minimal lethal dose).

Induction and elicitation of mice with S. aureus. Mice were infected by subcutaneous injection of 10^8 viable staphylococci once a week for 8 weeks. One week after the last injection with *S. aureus*, the sensitized mice were elicited twice, at 48 and 24 h before harvest of peritoneal cells, either by injection of two subcutaneous doses of 0.1 ml of staphylococcal bacteriophage lysate (Staphage Lysate [SPL], Delmont Laboratories, Inc., Swarthmore, Pa.) or by exposure in the chamber to aerosolization of 2 ml of the same lysate.

Preparation of peritoneal exudate cells. Peritoneal exudate cells were harvested without prior injection of irritants. For virus-immunized mice they were collected 2 weeks after exposure. For the bacterially induced animals they were collected 24 h after the second eliciting dose. The peritoneal cavity of each mouse was washed out with a total of 5 ml of cold Hanks balanced salt solution (Grand Island Biological Co., Grand Island, N.Y.) containing 10 U of heparin per ml (Liquaemin sodium, Organon Inc., Orange, N.J.). The exudate cells from each group of mice were pooled and washed three times with cold Hanks solution by centrifuging at 200 × *g* at 4 C for 10 min. The number of cells to be seeded onto cover slips was determined by the viable cell count. The number of viable monocytes in the washed cell suspension was determined by the trypan blue exclusion method in a hemacytometer.

Challenge of peritoneal cells with influenza virus. Peritoneal exudate cells collected at the appropriate times after immunization, as well as cells collected simultaneously from normal mice of the same age, were washed three times with Hanks solution before being challenged in vitro with the mouse-adapted swine strain. The washed cells suspended in Hanks solution supplemented with 10% fetal calf serum, 100 U of penicillin, and 100 μg of streptomycin per ml were challenged with the virus at a multiplicity of 10 EID_{50} per monocyte. After incubation at 37 C in a spinner culture flask for 60 min to allow for maximal viral adsorption, the infected cell suspensions were repeatedly washed in the cold in order to remove residual virus. On the first centrifugation, the packed cells were resuspended in 2 ml of cold Hanks solution containing 5% fetal calf serum and the antibiotics. This suspension was then transferred to a new centrifuge tube containing 40 ml of the same cold suspending medium and centrifuged. Resuspension in 40-ml volumes and centrifugation were repeated for a total of six times. It was found that little or no extracellular virus remained in the last wash solution, as determined by assays in embryonated chicken eggs. The washed infected cells were immediately suspended in Hanks solution containing 10% fetal calf serum and antibiotics, and 3×10^6 viable cells were introduced into each of a series of Leighton tubes with cover slips and placed at 37 C. At various time intervals, four cover slips with the adherent cells were removed; two were tested for development of hemad-

sorption and two for the development of S and V antigens by using indirect immunofluorescent (IF) techniques.

Hemadsorption test. For evaluation of hemadsorption the cells on the cover slips in the tubes were washed with cold Hanks solution and overlayered with 1 ml of a cold 0.5% suspension of guinea pig red cells, after which the tubes were placed at 4 C for 60 min. The cover slips were removed, washed and fixed in methanol, and stained with Giemsa. One thousand monocytes on duplicate cover slips were counted under light microscopy, and the number of monocytes with one, two to five, and more than five red cells attached were recorded.

Determination of S and V antigens by IF. Demonstration of the development of S and V antigens in the infected cells was accomplished by using the indirect IF test (20). For this purpose the cells on the other two cover slips were similarly washed with Hanks solution and on removal fixed with acetone. One-half of each pair of cover slips was tested for S and the other half for V antigen by using anti-S and anti-V guinea pig sera and fluorescein-tagged anti-guinea pig immunoglobulin G prepared in rabbits (Hyland Laboratories, Inc., Costa Mesa, Calif.). The production of pure anti-S and anti-V sera has been described (9, 10). One thousand cells on the two cover slips were counted, and the number showing fluorescence and the location of the staining were recorded.

The cells recorded in this study were chiefly mononuclear cells adherent to the cover slips. Our data give the percentages of total monocytes on cover slips after staining.

RESULTS

Growth of virus in normal mouse peritoneal cells: development of hemadsorption. Peritoneal cells collected from normal mice were infected in vitro with the mouse-adapted swine strain at a multiplicity of 10 EID_{50} per cell, as described.

An examination of infected cells immediately after seeding onto the cover slips, i.e., after the periods allowed for adsorption of virus, the washing of the cells to free them from residual virus and for attachment to glass, revealed no evidence of hemadsorption. This interval represented 1.5 h from the time of inoculation (Fig. 1A). With time, however, hemadsorption did appear and was most pronounced at 5 h (Fig. 1B). It remained at about the same level up to 18 h after inoculation, after which the experiment was discontinued.

The average percentage of peritoneal cells obtained from four experiments which were found with one, two to five, and more than five red cells attached at various time intervals after infection are given in Table 1. It was felt that the adsorption of five or more red cells to a large or small monocyte gave the most reliable evidence of the presence of virus in that cell. It can be seen that the number of peritoneal cells with five or more red cells attached was none at 1.5 h and an average of 9% (from 4 to 14%) after 5 h.

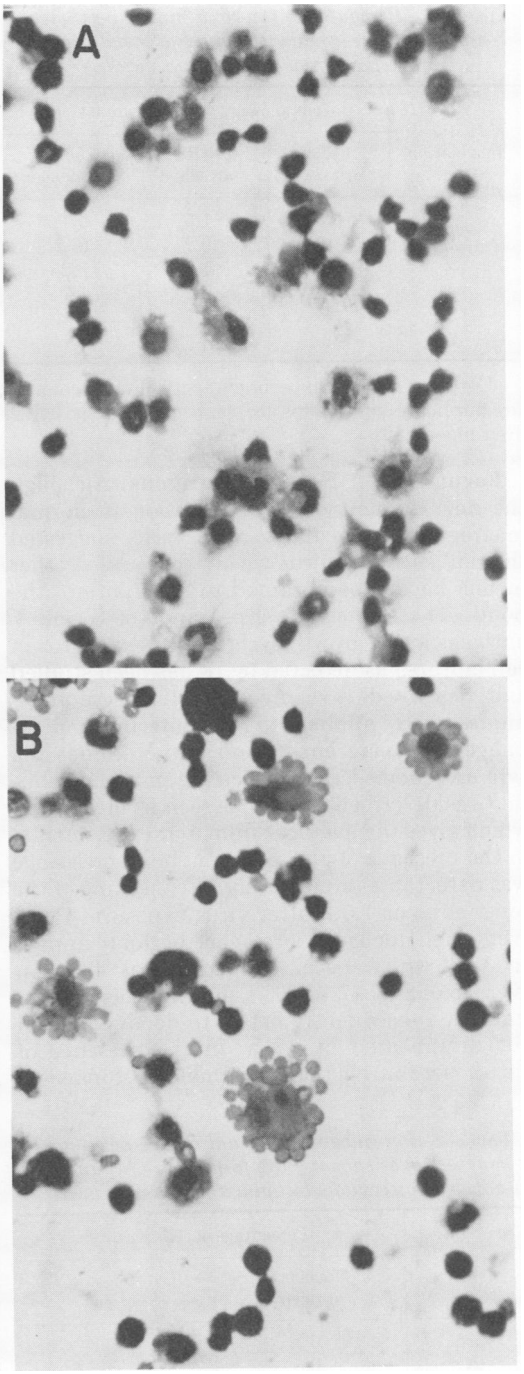

FIG. 1. *Photomicrograph of development of hemadsorption in normal mouse peritoneal macrophages after exposure to 10 EID_{50} of a mouse-adapted swine strain of influenza virus per cell. (A) 1.5 h after inoculation, (B) 5 h after infection. Magnification* $\times$ *340.*

TABLE 1. *Percentage of normal mouse peritoneal macrophages showing hemadsorption after inoculation with influenza virus*[a]

H after inoculation	Cells showing hemadsorption (%)		
	1 RBC attached	2-5 RBC attached	>5 RBC attached
1.5	3	1.5	0
2	2	0.7	0
3	5	1.8	0.25
4	4	2.2	1.7
5	4	3.3	9
18	5	3.5	6

[a] Average of four experiments, 1,000 cells counted on duplicate cover slips for each experiment. RBC, Red blood cell.

Formation of S and V antigens. Although the development of hemadsorption in normal macrophages inoculated with virus suggested the replication of virus within those cells, other means had to be obtained to substantiate the point. The sequential appearance of S and V antigens in the growth cycle of influenza virus in cell cultures as detected by IF has been studied and described (11). Accordingly, these techniques were applied to our infected macrophages by using anti-S and anti-V guinea pig sera and labeled anti-guinea pig immunoglobulin G as described. As can be seen from Table 2, which gives the averages from four experiments, in the presence of anti-S serum, no fluorescence was detectable immediately after seeding of the cover slips or 1.5 h after virus exposure. However, some nuclear fluorescence began to appear at 3 h, which became maximal at 4 h, an average of 14% (7 to 26%) showing this type of stain. It subsequently began to decline and all but disappeared by 18 h. In the presence of anti-V serum, on the other hand, cytoplasmic staining became detectable and reached its peak at 5 h, when 22% of cells (11 to 37%) showed fluorescence. This type of fluorescence was still visible in 17% of cells at 18 h. Although, in general, the sequential formation of S and V antigens in our infected macrophages correlates well with previous findings in cell cultures, our detection of V antigen in a small percentage of cells in the early hours is a discrepant observation. It is possible that it reflected the presence of adsorbed virus which remained on the cell surface and which was not detectable by hemadsorption or ingested whole virus within the cells which resisted uncoating. However, if the percentage of cells showing early cytoplasmic staining (less than 3 h) is subtracted from the percentage which showed the same staining at 5 h, about 17% of the monocytes produced V antigens. This figure correlates with the number found to produce S antigen.

TABLE 2. *Percentage of normal mouse peritoneal macrophages showing the formation of S and V antigens after inoculation with influenza virus*[a]

Time	Percentage of cells with	
	Nuclear S antigen[b]	Cytoplasmic V antigen
1.5	0	3
2	0.5	5
3	6	4
4	14	8
5	8	22
18	1	17

[a] Average of four experiments, 1,000 cells counted on duplicate cover slips for each experiment.

[b] No attempt was made to record the migration of S antigen into the cytoplasm after 4 h.

Figure 2A illustrates the detection of nuclear S antigen and Fig. 2B of cytoplasmic V antigen at 4 and 5 h, respectively, after infection. Obviously, the question of maturation of fully infective virus is important. However, we have thus far not obtained data on this point. Further study is planned.

Growth of virus in cells from virus-immunized mice. Growth of mouse-adapted swine virus in cells collected 2 weeks after immunization from mice immunized intraperitoneally or by aerosol with live egg-adapted swine virus was compared to growth in cells from normal mice, as revealed by the development of hemadsorption and the formation intracellularly of S and V antigens. In evaluating hemadsorption, only peritoneal cells with five or more red cells attached were taken as positive. Significantly fewer virus-challenged cells from immunized mice developed hemadsorption by 5 h as compared to those from normal mice, particularly when the cells were derived from aerosol-immunized animals (Fig. 3).

The results of the indirect immunofluorescent tests substantiated these findings. The percentages producing S antigen by 4 h or V antigen by 5 h were significantly lower in cells from virus-immunized mice (Fig. 4). Again, the cells from the aerosol-immunized mice were the least responsive and therefore most resistant to viral infection. Indeed, the immunofluorescent test data suggest that the cells from the aerosol-immunized mice were 9 to 10 times more resistant to infection than cells from normal mice.

Growth of virus in cells from mice induced and elicited with the bacterial system. Peritoneal cells from mice infected repeatedly with live *S. aureus* and elicited with SPL were

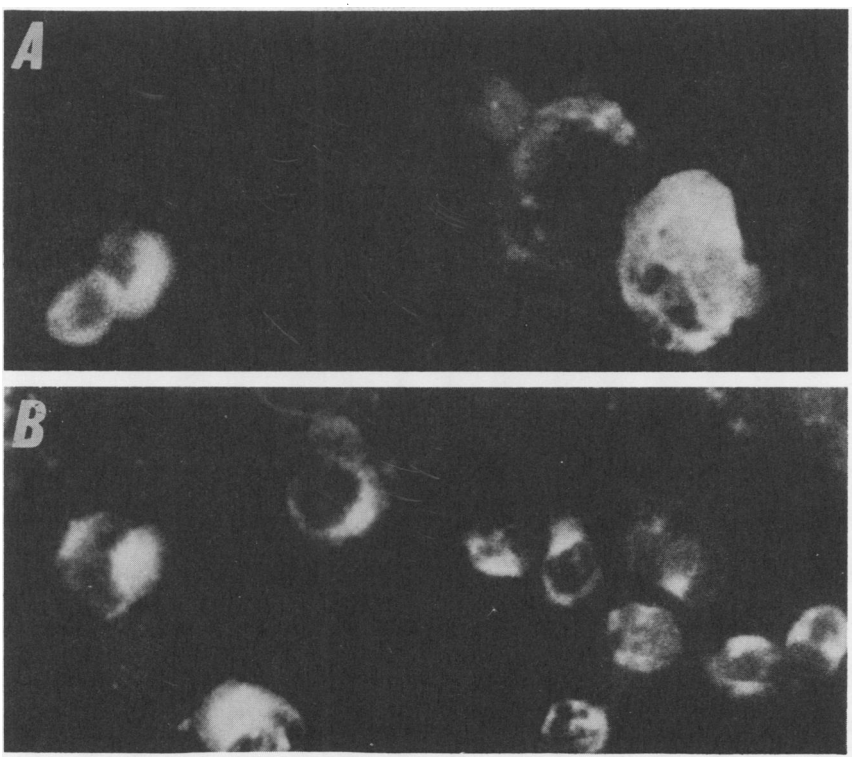

FIG. 2. *Photomicrograph of the appearance by IF of S and V antigens in normal macrophages inoculated with influenza virus. (A) S antigen in nuclei of small (left) and large monocytes (right) 4 h after infection. (B) V antigen in cytoplasm of large (left) and small monocytes (right) 5 h after infection. Magnification, ×500.*

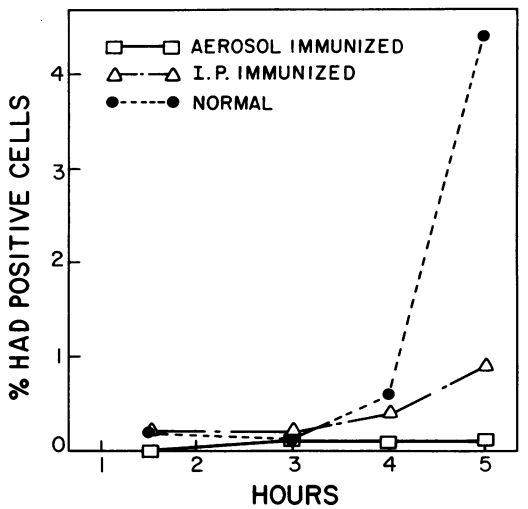

FIG. 3. *Percentage of peritoneal cells from virus immunized versus percentage of cells from normal mice which developed hemadsorption after virus challenge.*

similarly challenged in vitro with the same influenza strain along with cells from normal mice. The percentage which developed hemadsorption (five or more red cells attached) was markedly lower in cells derived from mice induced and elicited with the bacterial system than in cells from normal mice (Fig. 5). However, they were slightly more susceptible to the virus than cells from virus-immunized mice.

The results of the search for S and V antigens in these cells are shown in Fig. 6. The percentage containing S antigens in the nucleus at 4 h and V antigen in the cytoplasm at 5 h was also lower in cells from mice infected and elicited with the bacterial system. Roughly two to four times fewer cells from bacterially stimulated mice were apparently able to produce these antigens than cells from normal mice. Here also the cells from bacterially induced and elicited mice were slightly less inhibitory to the virus than the cells from virus-immunized mice.

Response of bacterially induced and elicited mice to in vivo challenge with influenza virus. Resistance to in vivo challenge with influenza virus could not be tested properly in virus-immunized animals because of the functioning of other immune mechanisms such as the development of specific circulating and secretory antibodies. However, mice infected with live *S. aureus* and elicited with SPL, when

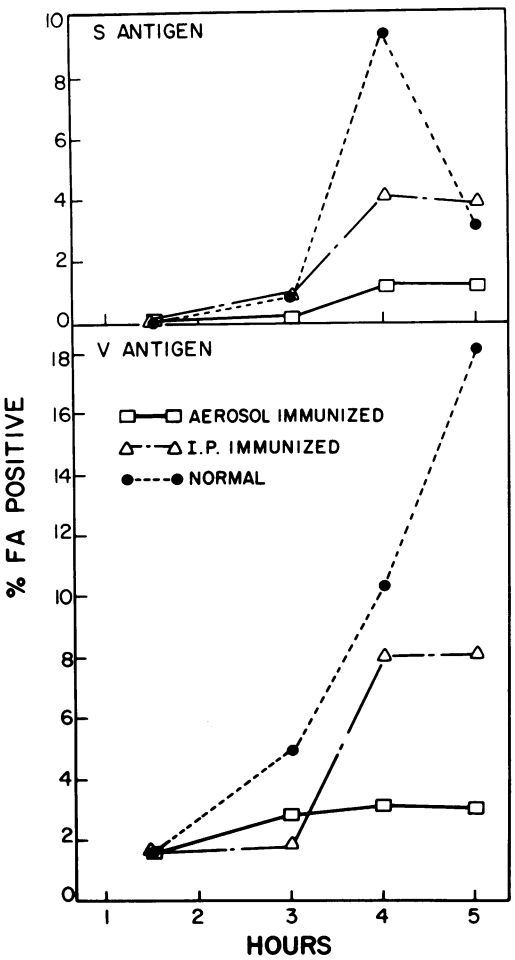

FIG. 4. *Percentage of peritoneal cells from virus immunized versus percentage of cells from normal mice which showed the formation of S and V antigens by IF after virus challenge.*

exposed to a minimal lethal dose of the mouse-adapted strain by aerosol, survived longer than normal mice. Figure 7 represents the average percentage of cumulative mortality observed in duplicate experiments; in each experiment, six mice per group were challenged. It can be seen that mice induced and elicited by the bacterial system, especially those mice which were elicited by aerosol, survived longer than normal mice after challenge with influenza virus. Although the mortality rate in these mice was also somewhat decreased, with the numbers of animals tested it could not be considered significant.

DISCUSSION

Although direct evidence was not sought in this work to differentiate between the mononuclear cells which remained adherent to glass, work by many investigators indicates that these

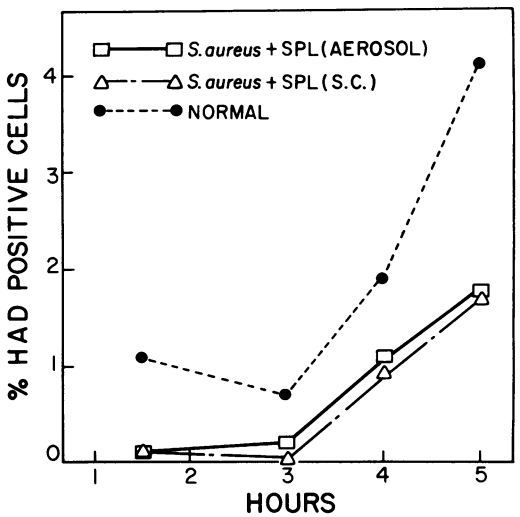

FIG. 5. *Percentage of peritoneal cells from mice infected with S. aureus and elicited with staphylococcal antigens versus percentage of cells from normal mice which developed hemadsorption subsequent to virus challenge.*

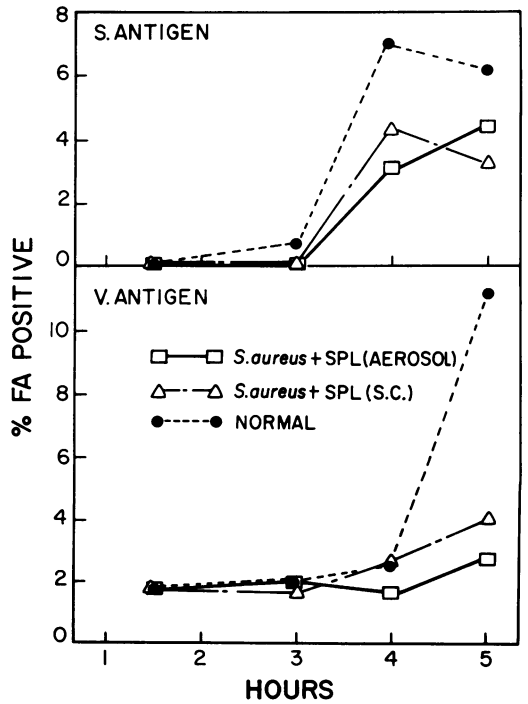

FIG. 6. *Percentage of peritoneal cells from mice infected with S. aureus and elicited with staphylococcal antigens versus percentage of cells from normal mice which showed the formation of S and V antigens by IF after virus challenge.*

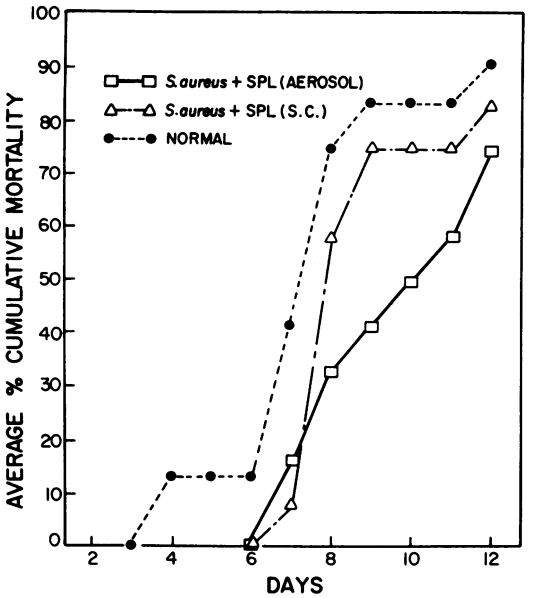

FIG. 7. *Average percent cumulative mortality (two experiments) in normal mice and in mice presensitized by the staphylococcal system after exposure by aerosol to a minimal lethal dose of influenza virus.*

cells may well have been predominantly macrophages. Therefore, the present study may be interpreted to have revealed that mouse-adapted influenza virus in contact with normal mouse peritoneal macrophages may be taken up by these macrophages and may replicate therein, as proven by development of hemadsorption and the sequential formation of S antigen in the nucleus followed by V antigen in the cytoplasm. These results could be significantly modified immunologically by two different means: (i) by specific immunization of the mice by exposure systemically or by aerosol to living homologous virus, and (ii) by nonspecific evocation of resistance by repeated infection of the mice with *S. aureus* followed by an eliciting administration of staphylococcal antigens (SPL) either subcutaneously or by aerosol.

In each case, the replication of virus in the macrophages was significantly inhibited, all but completely after aerosol immunization with live homologous virus, less completely by the *S. aureus*-SPL system. Precautions have been taken by extensive and protracted washing to exclude contamination of the macrophages by immunoglobulins in the peritoneal fluid or the presence of extracellular interferon. Production of antibody in vitro in the washed cells from the virus-immunized mice is unlikely because of the short duration of the experiments.

Lack of detectable circulating interferon in mice similarly induced and elicited with the bacterial system has been reported (17). Salvin et al. (15) demonstrated that BCG vaccination and OT elicitation evoked an interferon response. In the present work, although washed monocytes were used in vitro, the possibility of the presence of some intracellular interferon could not be excluded.

The finding of Hers (7) of bright nuclear and weak cytoplasmic fluorescence in a macrophage recovered from bronchial secretions of a human ill with influenza also suggests that viral replication in macrophages is not limited to an experimental model but may be a factor in the pathogenesis of human disease.

Previous investigators of cell-mediated resistance in relation to influenza virus have employed only killed virus for stimulation and inhibition of macrophage migration as their test system. Feinstone et al. (3) showed that, after the sensitization of their mice by injection into the footpads of killed mumps or influenza vaccines in complete Freund adjuvant, they could demonstrate the inhibition of migration of splenic lymphocytes taken from these mice in the presence of the homologous antigens. The inhibition was somewhat specific. Waldman et al. (19) demonstrated in the guinea pig that the stimulation of cellular resistance was route and dose dependent when a killed commercial influenza vaccine was employed as immunogen. When a dose comparable to that used for man (low dose group) was instilled intranasally, inhibition of macrophage migration became most pronounced in bronchial cells and was feeble in cells isolated from the spleen. The results were exactly reversed with respect to the source of lymphocytes when the mice were immunized systemically. When a significantly higher dose was given, these differences were almost obliterated. However, regardless of the dose, the development of humoral antibody reflected the route of administration, i.e., circulating neutralizing antibody was optimally stimulated by the systemic and secretory neutralizing antibody by the intranasal route.

In our studies using live virus, maximal stimulation of cellular resistance of the peritoneal macrophages occurred after the aerosol route. Under these conditions, of course, virus had the optimal opportunity to multiply and to provide a large antigenic mass for induction of cell-mediated resistance. It was found in an earlier study that mice similarly exposed to the same or other strains of influenza virus developed high levels of circulating antibodies (F. S. Lief and L. Syrucek, unpublished data).

Little is known as to the nonspecific elicitation of cell-mediated resistance with respect to influenza. Myrvik (13) studied nonspecific elici-

tation of cell-mediated resistance among different bacterial groups. He used stimulation of the hexose monophosphate shunt as the indicator reaction. He found BCG vaccination effective in activating the pulmonary macrophages of rabbits and protecting against listerial infection but not against *Pasteurella tularensis*, which is cytotoxic.

In the present study it was not determined whether the resistance of our virus or bacterially stimulated macrophages derived essentially from activation of the intracellular cidal mechanisms, as demonstrated in the investigations of Dannenberg (2, 18) and Mackaness (12), or were complemented by continuing elaboration and liberation of interferon, as demonstrated by Glasgow (4), or perhaps other mechanisms. The web of events resulting from interaction of competent peritoneal cells with challenge virus is complex and in need of further study.

Our observations in vivo on survival after challenge with the swine influenza virus probably do not represent optimal conditions. Although there was some evidence of protection as revealed by increased survival time, perhaps the conditions of exposure and evaluation were not sufficiently sensitive. Possibly exposure to a less lethal concentration of virus and evaluation by lung lesions and/or quantitation of the amount of virus produced would yield more conclusive results.

The findings reported here strengthen the position that the development and functioning of cell-mediated resistance must be considered in evaluating the interaction between a virus, specifically influenza virus, and its intact host.

ACKNOWLEDGMENTS

This investigation was supported in part by World Health Organization grant Z2/181/20.

LITERATURE CITED

1. Allen, E. G., and S. Mudd. 1973. Protection of mice against vaccinia virus by bacterial infection and sustained stimulation with specific bacterial antigens. Infect. Immunity **7**:62-67.
2. Dannenberg, A. M., Jr. 1968. Cellular hypersensitivity and cellular immunity in the pathogenesis of tuberculosis: specificity, systemic and local nature and associated macrophage enzymes. Bacteriol. Rev. **32**:85-102.
3. Feinstone, S. M., E. H. Beachey, and M. W. Rytel. 1969. Induction of delayed hypersensitivity to influenza and mumps virus in mice. J. Immunol. **103**:844-849.
4. Glasgow, L. A. 1970. Cellular immunity in host resistance to viral infections. Arch. Intern. Med. **126**:125-134.
5. Gresser, I., and D. J. Lang. 1969. Relationships between viruses and leucocytes. Progr. Med. Virol. **8**:82-130.
6. Henle, W., and G. Henle. 1949. Studies on host-virus interactions in the chick embryo-influenza virus system. II. The propagation of virus in conjunction with the host cells. J. Exp. Med. **90**:13-37.
7. Hers, J. F. P. 1963. Fluorescent antibody technique in respiratory viral diseases. Amer. Rev. Resp. Dis. **88**:316-337.
8. Kapral, F. A., and M. G. Shayegani. 1959. Intracellular survival of staphylococci. J. Exp. Med. **110**:123-138.
9. Lief, F. S. 1963. Antigenic analysis of influenza viruses by complement fixation. VII. Further studies on production of pure anti-S serum and on specificity of type AS antigens. J. Immunol. **90**:172-177.
10. Lief, F. S., and W. Henle. 1959. Methods and procedures for use of complement-fixation technique in type- and strain-specific diagnosis of influenza. Bull. World Health Org. **20**:411-420.
11. Löffler, H., G. Henle, and W. Henle. 1962. Attempts to influence the incomplete reproductive cycle of influenza virus in HeLa cells by antibodies. J. Immunol. **88**:763-776.
12. Mackaness, G. B. 1970. The mechanism of macrophage activation. *In* S. Mudd (ed.), Infectious agents and host reactions. W. B. Saunders Co., Philadelphia.
13. Myrvik, Q. N. 1972. Function of the alveolar macrophage in immunity. J. Reticuloendothel. Soc. **11**:459-468.
14. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent end points. Amer. J. Hyg. **27**:493-497.
15. Salvin, S. B., J. S. Younger, and W. H. Lederer. 1973. Migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. Infect. Immunity **7**:68-75.
16. Shayegani, M., S. J. DeCourcy, Jr., and S. Mudd. 1973. Cell-mediated immunity in mice infected with *S. aureus* and elicited with specific bacterial antigens. J. Reticuloendothel. Soc. **14**:44-51.
17. Shayegani, M., and S. Mudd. 1973. Lack of detectable circulating interferon in mice protected against vaccinia virus by induction and elicitation with bacterial systems. Infect. Immunity **7**:117-118.
18. Shima, K., A. M. Dannerberg, Jr., M. Ando, S. Chandrasekhar, J. A. Seluzicki, and J. I. Fabrikant. 1972. Macrophage accumulation, division, maturation, and digestive and microbicidal capacities in tuberculous lesions. I. Studies involving their incorporation of tritiated thymidine and their content of lysosomal enzymes and bacilli. Amer. J. Pathol. **67**:159-176.
19. Waldman, R. H., C. S. Spencer, and J. E. Johnson III. 1972. Respiratory and systemic cellular and humoral immune response to influenza virus vaccine administered parenterally or by nose drops. Cell. Immunol. **3**:294-300.
20. Weller, T. H., and A. H. Coons. 1954. Fluorescent antibody studies with agents of varicella and herpes zoster propagated *in vitro*. Proc. Soc. Exp. Biol. Med. **86**:789-794.