EXPERIMENTAL TRANSMISSION OF INFLUENZA VIRUS INFECTION IN MICE

III. Differing Effects of Immunity Induced by Infection and by Inactivated Influenza Virus Vaccine on Transmission of Infection*

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Numerous epidemiologic (1–4) and experimental (5–10) studies have shown that the presence of type-specific antibody induced either by previous infection or by artificial immunization with inactivated vaccine is associated with protection of subjects against the pathologic consequence of infection with influenza virus of the same subtype. Other investigations (8–13) have provided evidence of a less striking heterotypic protective effect in subjects with antibody to one subtype challenged with influenza virus of a different subtype.

Recent experiments in this laboratory have confirmed the presence of double antigenicity in a plaque purified, stable recombinant virus prepared from A_0 and A_2 virus parents. Mice immunized by infection with this recombinant virus have hemagglutination inhibiting antibody only against the A_0 virus parent but are equally protected against subsequent A_0 and A_2 virus challenges as judged by reduction in virus replication in the lungs and prevention of lung lesions. The broadened immunity induced by infection with this hybrid virus affords less protection than the homotypic immunity elicited by prior infection with influenza virus of the same subtype as the challenge virus, but is more effective in inhibiting viral replication and preventing lung lesions than the slight heterotypic protection observed when mice are immunized by infection with virus of one subtype and are challenged with influenza virus of a different subtype (14).

There is evidence which suggests that one manifestation of immunity to influenza virus infection is a decreased likelihood of infection (as shown by antibody rise) in immune subjects compared to nonimmune subjects under similar circumstances of exposure (4–7). Nevertheless, infection with a subsequent rise

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in antibody titer has been clearly and repeatedly shown to occur in individuals with preexisting homotypic or heterotypic antibody to the infecting influenza virus (4–7, 15). Virtually no information exists, however, as to whether these partially immune subjects are as capable of transmitting infection as subjects lacking specific antibody.

An experimental model designed to study the transmission of influenza virus infection in mice (16) was employed in the present experiments to investigate the effects of varying methods of immunization on transmission of infection.

Materials and Methods

Mice.—Manor Farms (MF-1) specific pathogen-free male mice 10–16 wk of age were employed in all experiments.

Lungs were removed aseptically at designated intervals and ground in glass tubes in accordance with techniques previously described (17).

Viruses.—The Stuart-Harris neurovirulent strain of WS virus (NWS) was employed as an infective strain of influenza A_0 virus, and the PR8 strain of A_0 virus was used as formalin-inactivated vaccine (400 chick cell agglutinating units/cc). An unadapted, inhibitor-sensitive strain of virus isolated at the Rockefeller University, N. Y., ($RI/5^+$) (18) and mouse adapted Jap. 305 virus were used as infective A_2 viruses. An unadapted strain of Jap. 305 virus (200 chick cell-agglutinating (CCA) units/cc) was employed as formalin-inactivated A_2 vaccine. In most experiments the Lee strain of influenza B virus was also used.

One other virus employed in most of these experiments is a recombinant virus X-7 derived from the NWS strain of A_0 and the $RI/5^+$ strain of A_2 virus. This virus has an A_0-like hemagglutinating antigen and a minor A_2-like antigen demonstrable by complement fixation (CF) (19) and plaque size reduction techniques (19, 20). At least part and probably all of the A_2 antigen is neuraminidase (21).

Demonstration and Titration of Virus.—The presence of virus in the lungs of animals exposed to transmitted infection and the titers of virus in the lungs of infector mice were determined by methods previously described (16).

Hemagglutinating Inhibiting (HI) Antibody Titrations.—HI antibody was titrated in individual mouse sera 4 wk after immunization and just prior to A_2 virus challenge. The mouse-adapted A_2 (Jap. 305) virus was used as the antigen, and in preliminary tests with this virus it was found that trypsin or periodate treatment of serum was not necessary. Sera were heated at 56°C for 30 min and then serial 2-fold dilutions of 0.2 cc of heated serum were made in phosphate-buffered saline (PBS). A 0.2 cc amount of the mouse-adapted Jap. 305 virus containing 16–32 hemagglutinating units was added to each tube. Then, 0.4 cc of human "O" red cells were added an after 50 min at room temperature the tubes were observed for the absence or presence of agglutination.

Scoring of Pulmonary Lesions.—A modification of the maximal score method (22) was used, in which the extent of pulmonary lesions was expressed as a percentage of the total lung surface.

Aerosol Procedure.—The apparatus and technique used to generate an aerosol of infective virus has been described elsewhere (16). Mice were exposed during a 30 min period to an estimated 10–100 mouse infective doses (MID_{50}) of each of the viruses employed.

Contact Procedure.—Immediately after initiation of infection in the aerosol chamber, infector mice were placed in small stainless steel cages, two mice per cage. 24 hr later two previously uninfected mice were placed in each of the cages. After a 24 hr period of contact the previously uninfected mice were removed and were isolated for 48 hr prior to testing their lungs for the presence of infective virus.

Immunization Procedures.—(See Table I). In all of the present studies, mice were challenged with mouse-adapted A_2 (Jap. 305) influenza virus 4 wk after immunization. Challenge was presented in the form of either an artificial aerosol of virus or by exposure to infection transmitted from other animals infected with the A_2 virus. Mice were immunized either with homotypic (A_2) or heterotypic (A_0) virus by aerosol infection or by intraperitoneal inoculation of formalin-inactivated virus. Control mice were given saline intraperitoneally, or were exposed to aerosols of heterologous influenza B virus or to saline aerosols. The effects of immunization on infector mice were assessed in terms of pulmonary virus titers and lung lesions after A_2

TABLE I

Effect of Various Immunization Procedures on Transmission of Influenza A_2 Virus Infection in Mice—Experimental Design

Infector mice

Immunization	Challenge	Measurements	
		Infector mice	Contact mice
A_2 infection	A_2 aerosol	Pulmonary Virus titers (48 hr)	Per cent of contacts infected after exposure to each infector group
A_0 infection	A_2 aerosol		
*A_2 i.p.	A_2 aerosol	Lung lesions (7 days)	
‡A_0 i.p.	A_2 aerosol		
X-7 infection	A_2 aerosol		

Contact mice

Immunization	Challenge	Measurements
A_2 infection A_0 infection *A_2 i.p. ‡A_0 i.p. X-7 infection	Exposure to infector mice infected with A_2 virus	Per cent of each contact group infected after contact exposure

* Formalin-inactivated Jap. 305 virus 200 CCA units/cc.

‡ Formalin-inactivated PR8 virus 400 CCA units/cc.

challenge, and by their ability to transmit infection to exposed contacts. In contact animals the effect of immunization was judged simply by the proportion of each contact group which acquired transmitted infection.

EXPERIMENTAL RESULTS

Effect of Immunity Induced by Prior Infection.—Mice were infected by exposure to aerosols of A_2, A_0, or recombinant X-7 (A_0A_2) virus. Control mice were infected with influenza B virus or were exposed to saline aerosols. 4 wk later the animals were challenged by exposure to an aerosol of influenza A_2 (Jap. 305) virus. Pulmonary virus was titrated 48 hr later and lung lesions were assessed 7

days after infection. The results are shown in Table II. Mice immunized 4 wk previously by homotypic influenza A_2 virus infection were competely refractory to reinfection. None had demonstrable pulmonary virus 48 hr after challenge. Mice immunized by infection with the heterotypic influenza A_0 virus were partially protected as shown by lower pulmonary virus titers and less extensive lung lesions than control mice. Immunization by infection with the recombinant X-7 virus was more effective than immunization by infection with the A_0 parent, and resulted in even lower pulmonary virus titers and less extensive lung lesions, but the protection afforded was not as great as that induced by the A_2 virus parent (homotypic to the challenge). Prior infection with the heterologous influenza B virus provided no protection against the A_2 virus challenge.

TABLE II

Effect of Previous Infection of Mice with Homotypic or Heterotypic Virus on Subsequent Challenge with Influenza A_2 Virus

Initial infection	*HI antibody to A_2 virus	*Challenge infection	‡Pulmonary virus titers (48 hr)	Lesions (7 days)
				%
Saline	<1:8	A_2	7.7	61.3
B (Lee)	<1:8	A_2	7.7	62.5
A_0 (NWS)	<1.8	A_2	6.5	30
A_0A_2	<1.8	A_2	5.5	9.6
A_2 (RI/5+)	1:32	A_2	<1.0	0

* 4 wk following initial infection.

‡ Log_{10}, EID_{50}, mean of individual titers, five animals in each group.

The effects of these differing immunization procedures on transmission of infection were studied in cohort mice immunized at the same time. Some animals were challenged by exposure to an artificial aerosol of A_2 virus and were employed as infectors with normal contact mice. Others were placed in contact with unimmunized infector mice infected 24 hr earlier with influenza A_2 virus. The summarized results of eight experiments are presented in Table III. The upper part of the table indicates the results when immunized mice, challenged with A_2 virus were used as infectors; the lower part of the table indicates the results when immunized mice were used as contacts with unimmunized A_2 infectors. Mice immunized by prior homotypic influenza A_2 virus infection were not reinfected when challenged and did not transmit infection. Similarly as shown in the lower half of the table, they were completely refractory to infection transmitted by "control" (previously unimmunized) infectors. Infector mice immunized by prior infection with influenza A_0 virus or with the X-7 virus transmitted infection less frequently than control infectors, and contact mice immunized 4 wk

earlier by infection with these viruses acquired transmitted infection less frequently than control contacts. In each case the effect was more pronounced in mice immunized with the recombinant (X-7) virus. Thus with the experimental conditions employed, mice infected with A_2 (RI/5+) virus were completely refractory to reinfection when challenged either by exposure to an aerosol of A_2 virus or by exposure to A_2 virus infection transmitted by other mice. The partial protection afforded by prior infection with influenza A_0 virus or recombinant (A_0A_2) virus is associated with decreased transmission during their infection to A_2 virus and with diminished susceptibility to A_2 virus infection transmitted by other mice.

TABLE III

Effect of Previous Infection of Mice with Homotypic, Heterotypic, or Heterologous Virus on Transmission of Influenza A_2 Virus Infection

Previous influenza virus infection		Contacts infected	
Infector* group	Contact group		
			%
Saline	None	81/162	(50.0)
B (Lee)	None	57/161	(35.4)‡
A_0 (NWS)	None	15/145	(10.5)
A_0A_2 (X-7)	None	9/96	(9.4)
A_2 (RI/5+)	None	0/40	(0)
None	Saline	37/75	(49.3)
None	B (Lee)	26/64	(40.6)‡
None	A_0 (NWS)	13/49	(26.5)
None	A_0A_2 (X-7)	(7/53)	(13.2)
None	A_2 (RI/5+)	(0/40)	(0)

* Aerosol infection 4 wk prior to A_2 virus challenge.

‡ $P > 0.05$.

Effect of Immunization with Inactivated A_2 Virus.—Mice were immunized by a single intraperitoneal injection of 0.2 cc of a 1:5 dilution of formalin-inactivated A_2 (Jap. 305) virus containing 200 CCA units/cc. Control mice were given saline intraperitoneally. 4 wk later some mice from each group were bled and their sera tested for HI antibody against A_2 (Jap. 305) virus. The remaining mice were challenged with A_2 (Jap. 305) virus and pulmonary virus titers were measured 48 hr later and lung lesions were assessed 7 days later. The results as seen in Table IV simply indicate that mice immunized with inactivated A_2 vaccine in this dosage have HI antibody at the time of A_2 virus challenge and have lower pulmonary virus titers and less extensive lesions following challenge. It should be noted that HI antibody titers following intraperitoneal injection of inacti-

vated A_2 vaccine were equivalent to those induced by prior A_2 virus infection (Table II). The effects on transmitted infection induced by immunization with inactivated homotypic (A_2) virus were studied as follows: mice were inoculated intraperitoneally with inactivated A_2 virus or with saline. 4 wk later some mice

TABLE IV

Effect of Prior Inoculation with Inactivated A_2 Virus Vaccine on Subsequent A_2 Virus Challenge

Immunization	ΔA_2 i.p.	Saline i.p.
HI antibody to A_2 virus*	1:32	<1:8
Challenge*	A_2 (aerosol)	A_2 (aerosol)
Pulmonary virus titers (48 hr)‡	5.7	7.7
Lung lesions (7 days) %	2.5	62

Δ 0.2 cc of a 1:5 dilution of formalin-inactivated Jap. 305 virus 200 CCA units/cc.

* 4 wk after immunization.

‡ EID_{50}, $\log_{10}$, individual titers of five animals in each group.

TABLE V

Effect of Inactivated Homotypic Vaccine on the Transmission of Influenza Virus Infection in Mice

Infector mice	Contact mice		
	No. infected/total No. in group		
	Immunized	Unimmunized	Total
Immunized*	6/32 (18.7%)	30/60 (50%)	36/92 (39.1%)
Unimmunized	2/31 (6.4%)	29/61 (47.5%)	31/92 (33.7%)
Total	8/63 (12.7%)	59/121 (48.8%)	

* 0.2 cc of a 1:5 dilution of formalin-inactivated A_2 virus, intraperitoneally 4 wk before challenge.

from each group were infected with A_2 virus and were used as infectors, while the remaining animals were employed as contacts.

Four different contact situations thus were established: immunized infectors and immunized contacts; immunized infectors and unimmunized contacts; unimmunized infectors and immunized contacts; and unimmunized infectors and unimmunized contacts. The proportion of contacts infected in each contact situation can be seen in Table V. Immunized contacts acquired transmitted in-

fection far less frequently than unimmunized contacts. However, immunized infectors transmitted infection just as readily (39.1%) as unimmunized infectors (33.7%). Therefore, although immunized infectors had lower pulmonary virus titers following A_2 virus challenge than unimmunized infectors, their ability to transmit infection was not affected.

Effect of Inactivated Heterotypic (A_0) Vaccine.—Mice inoculated weekly for 3 wk with saline or with 0.2 cc of a 1:5 dilution of formalin-inactivated A_0 virus (400 CCA units/cc) were challenged with A_2 virus 1 wk after the last injection and were used as infectors, or were not challenged and were used as contacts. The results can be seen in Table VI. Inactivated A_0 virus given intraperitoneally did not result in lower pulmonary virus titers following A_2 challenge and infectors immunized in this way were as capable of transmitting A_2 virus infec-

TABLE VI

Effect of Parenteral Immunization with Inactivated Influenza A_0 Virus on Pulmonary Virus Titers and Transmission of Infection Following Influenza A_2 Virus Challenge

Infector mice		Contact mice/No. infected		
Immunization	Pulmonary virus titers (48 hr)*	Saline	ΔA_0	Total
Saline	7.5	6/10	4/10	10/20
Δ A_0	7.6	5/10	3/10	8/20
Total.....		11/20	7/20	

Δ 3 intraperitoneal injections at weekly intervals 0.2 cc of a 1:5 dilution of formalin-inactivated PR8 virus 400 CCA units/cc.

* Log_{10} EID_{50} five animals in each group.

tion as unimmunized infectors. Similarly, contact mice immunized with inactivated A_0 virus were just as susceptible to transmitted A_2 virus infection as unimmunized contacts. Therefore, inactivated A_0 virus vaccine given at a peripheral site did not protect mice against A_2 virus challenge and did not influence either the ability of immunized infectors to transmit infection or the susceptibility of immunized contacts to transmitted infection.

DISCUSSION

The definitive expression of antiviral immunity is the capacity of the host to inhibit multiplication of the invading virus and consequently to inhibit virus-induced lesions, but an alternative expression is the ability of the host to resist the initiation of infection under circumstances of exposure in which infection is likely. From an epidemiologic standpoint, still another consideration assumes importance — the capacity of a partially immune (but infectable) host to trans-

mit infection to others. In the present experiments, immunity induced to influenza A_2 virus by different immunization procedures was assessed in three ways: (*a*) in terms of its protective effect in mice directly challenged with aerosols of influenza A_2 virus; (*b*) by its effect on susceptibility to initiation of mouse-to-mouse transmitted infection; and (*c*) by its effect on the capacity of immunized infector mice to shed virus and to transmit infection to other mice. The effects of the different immunization procedures as reflected by these three indications of altered host susceptibility are summarized in Table VII. All of the changes observed are believed to have been mediated through specific immunologic mechanisms. Viral interference has been excluded as a factor because of the duration of altered host susceptibility and because of the absence of any effect following heterologous influenza B virus infection (17).

TABLE VII

Summary of Effects of Differing Immunization Procedures on Response to Challenge Infection, Susceptibility to Transmitted Infection, and the Capacity to Transmit Influenza A_2 Virus Infection

Immunization	Virus titers and lesions following aerosol challenge	Resistance to mouse-to-mouse transmitted infection	Capacity to transmit challenge infection
A_2 infection	No infection	Complete	No transmission
A_2 vaccine*	Reduced	Increased	No effect
A_0 infection	Reduced	Increased	Decreased transmission
A_0A_2 (X-7) infection	Reduced	Increased	Decreased transmission
A_0 vaccine	No effect	No effect	No effect
B infection	No effect	No effect	No effect

* Intraperitoneal injection of noninfective virus.

With the exception of prior parenteral inoculation of inactivated heterotypic influenza A_0 virus all of the immunization procedures utilizing Type A influenza viruses resulted in at least partial protection of mice challenged by exposure to nebulized influenza A_2 virus. This protection was reflected by a reduction in pulmonary titers of challenge virus and by diminished lung lesions. The most potent immunization procedure was prior infection with homotypic influenza A_2 virus. Mice immunized in this way were not reinfected when challenged with as much as 1000 MID_{50} of aerosolized virus. This refractoriness to aerosol challenge has been found in other experiments in this laboratory to persist for at least 1 yr. In contrast, mice immunized with a single intraperitoneal injection of *inactivated* (noninfective) influenza A_2 virus were uniformly infected when challenged by exposure to an aerosol of 100 MID_{50} of A_2 virus. The decreased protection afforded by inactivated homotypic vaccine cannot be explained on the

basis of inadequate antibody response as the serum titers of hemagglutinating-inhibiting antibody in the completely resistant mice immunized by homotypic infection and in mice immunized by inactivated homotypic vaccine were identical. The data, therefore, suggest that local immunologic mechanisms are operative. Francis (23), and Fazekas de St. Groth (24) have shown that the extent to which mice are protected against influenza virus challenge is more closely correlated with titers of antibody in respiratory tract secretions than with titers of humoral antibody. It is thought that the local antibody is derived from humoral antibody which diffuses into the respiratory tract secretions from the blood stream, but an alternative hypothesis is that the antibody is produced directly by cells within or adjacent to the respiratory tract. A similar mechanism has been postulated to explain the resistance to gastrointestinal reinfection observed in subjects immunized with live attenuated poliovirus that is not observed in subjects immunized with inactivated poliovirus vaccine (25). Recent studies have shown that the immunologically specific inhibitory activity of respiratory tract secretions resides predominantly in the γA-globulin fraction of the proteins recovered whereas the γG-globulin fraction contains most of the serum activity. It may be that infection provides a more potent stimulus to the formation and/or the release of γA-globulin in respiratory tract secretions (26–28).

Additional evidence that protection is not due to preexisting humoral antibody alone is provided by the observation that mice immunized by infection with the heterotypic influenza A_0 or A_0A_2 viruses (although lacking detectable serum influenza A_2 antibody) were partially immune. Following influenza A_2 virus challenge by aerosol these mice had lower pulmonary virus titers and less extensive lung lesions than control animals.

The effects of the different immunization procedures on the likelihood of immunized contact mice acquiring transmitted infection were exactly parallel to resistance to aerosol challenge with influenza A_2 virus. All of the immunization procedures employed, with the exception of inactivated heterotypic A_0 virus vaccine, resulted in resistance of mice to mouse-to-mouse transmitted infection. Mice immunized by A_2 infection that were refractory to reinfection by nebulized aerosol challenge were also completely refractory to A_2 virus infection transmitted by other mice, whereas 12.7% of contacts immunized with inactivated A_2 virus vaccine acquired transmitted infection when exposed to infected cage mates. Similarly, immunization by prior infection with A_0 or A_0A_2 viruses (associated with increased resistance to nebulized A_2 virus challenge) resulted also in an increased resistance to the likelihood of acquisition of mouse transmitted infection.

With respect to the effect of these immunization procedures on the capacity of infector mice to transmit infection, locally expressed immunologically specific factors again seem to be operative. It is obvious that the complete refractoriness

to A_2 virus reinfection in mice immunized by prior homotypic (A_2) infection renders them incapable of transmitting infection. In contrast, mice immunized by parenteral injection of inactivated homotypic A_2 virus vaccine could be reinfected by exposure to aerosols of nebulized virus and were fully capable thereafter of transmitting infection to other mice. This unimpaired transmission is difficult to explain in that mice immunized in this way had lower titers of pulmonary virus than control infectors and presumably had less virus available to be shed into the environment. It may be that the virus which is shed during transmission is derived from the most superficial portions of the respiratory epithelium where it is less vulnerable to inactivation by serum antibody. Conversely, mice partially immunized by prior infection with the heterotypic influenza A_0 or recombinant A_0A_2 viruses transmitted infection less well following their infection with A_2 virus, although peak pulmonary virus titers were as high or higher than in animals immunized with inactivated A_2 virus vaccine. The immunological specificity of these effects on the transmission of infection to other mice is suggested by the observation that transmission of A_2 virus infection is not altered in mice previously infected with the antigenically unrelated influenza B virus. It may be that these local immunologic mechanisms affect the availability of unbound infectious virus for expulsion into the environment.

The superiority of infection-induced immunity both in its effect on susceptibility to challenge and in its effect on the shedding of virus and the subsequent spread of infection may have important epidemiologic implications. Similarly, the broadened immunity induced by infection with a hybrid influenza virus possessing antigenic components of both parents has potential value as an immunization procedure.

SUMMARY

Immunization of mice by infection or intraperitoneal injection with homotypic A_2, heterotypic A_0, or recombinant A_0A_2 virus have differing effects on transmission of influenza A_2 virus infection. Immunization by infection with A_2 virus resulted in refractoriness to reinfection either by artificial aerosols or by exposure to infected cage-mates. Immunization by inoculation with inactivated A_2 virus vaccine resulted in a decreased susceptibility to transmitted infection in immunized contacts, but following A_2 virus challenge, transmission of infection by immunized infectors was not altered. Immunization by infection with influenza A_0 virus or recombinant A_0A_2 virus resulted in a decreased susceptibility to transmitted A_2 virus infection in immunized contacts, and to decreased transmission after A_2 virus infection in immunized infector mice. These differing effects on transmission of infection are attributed to differences in specific local immunologic responses following the various immunization procedures.

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