NEUTRALIZATION TESTS WITH SERA OF CONVALESCENT OR IMMUNIZED ANIMALS AND THE VIRUSES OF SWINE AND HUMAN INFLUENZA

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Smith, Andrewes, and Laidlaw (1, 2) have noted that the immunity which follows infection of ferrets with either swine or human influenza virus confers a considerable active resistance against the other virus. They further observed, in neutralization tests done in ferrets, that, while each strain was neutralized by admixture with homologous ferret immune serum, neutralization by the heterologous serum was inconstant. In addition, Shope (3) has reported that mice immune to either the swine or human influenza virus resist later infection with the heterologous strain. On the other hand, Francis (4) was unable to demonstrate neutralizing antibodies against the P. R. 8 strain of the human influenza virus in the serum of swine immune to swine influenza. These facts suggest that the active immunity which develops in animals, following infection with either the swine or human influenza virus, is effective against both viruses. Nevertheless, it seems certain that the sera of such animals, although uniformly neutralizing the homologous virus, may or may not neutralize the heterologous virus.

The present experiments were carried out in an effort to determine the factors involved in the development of heterologous virus-neutralizing antibodies following infection or immunization with the swine or human influenza virus. It was hoped that the information obtained would be useful in interpreting the results of experiments in which samples of human serum were tested in duplicate for their ability to neutralize the human and swine influenza viruses (5, 6).

Materials and Methods

Strains of Virus.—The viruses employed in the present experiments were the P.R.8 strain human influenza virus isolated by Francis in 1934 (7) and the strain 15 swine influenza virus obtained by Shope in Iowa in 1930. Both were well adapted to mice and killed them quite regularly in less than 6 days following infection.

Sera.—The 2 horse sera used were prepared in England (8) and obtained through the courtesy of Drs. Laidlaw, Smith, Andrewes, and Dunkin. All other sera were from animals studied in our laboratories. The ferrets infected with the Alaska strain and the swine infected with the P.R.8 strain were employed in experiments the details of which have not yet been published.

Neutralization Tests.—The tests with swine influenza virus were performed as follows:

Weighed amounts of glycerolated infected mouse lung were ground with sand and suspended in physiological saline to form a 2 per cent suspension.

The suspension was allowed to sediment for 10 minutes, and at the end of this time the supernatant fluid was removed by pipette and used as the source of virus. Equal parts of the serum to be tested and the virus were mixed and stored for 2 hours in the refrigerator (4°C.) prior to administration intranasally to the test mice. The mice were lightly etherized and their noses and mouths were then immersed in the serum-virus mixture contained in one side of a slightly tilted Petri dish, as described in a previous paper (3). 4 mice were employed for each test in most instances. On the 6th day after inoculation, at which time the control mice were either dead or very ill, the remaining mice were killed with chloroform and their lungs removed. The neutralizing effect of a serum of unknown potency upon the virus was measured by comparing the extent of the pulmonary lesions in mice receiving a mixture of that serum and virus with the lesions in control mice receiving the virus and normal serum.

The procedure employed in tests made with human influenza virus differed in certain particulars from that outlined above. A centrifugalized 10 per cent suspension of infected mouse lung was used; the serum-virus mixtures were incubated at 37°C. for 30 minutes; and 0.03 cc. of the mixture was given to each mouse. The procedure is described in detail in the following paper (5). Although the exact procedures differed in the tests with swine virus and human virus, nevertheless, a sufficient number of tests with the same virus and the same serum were done in duplicate by the two methods to indicate that the results obtained are closely comparable.

Results of the Neutralization Tests

The results of experiments in which sera from animals immune to either the human or swine influenza virus were tested for their ability to neutralize the two viruses are outlined in Table I.

As shown by the data in Table I, the human and the swine influenza viruses were consistently neutralized by their homologous immune sera. Convalescent serum from animals submitted to but a single virus exposure appeared to be as efficient in this respect as that from animals submitted to repeated virus insults. On the other hand, after repeated inoculations of one virus, the serum of the animal was frequently found to exert some effect against the heterologous virus. This was more marked in the cases of animals receiving human influenza virus than in those receiving swine virus. The conditions involved are not strictly comparable, however, because multiple inoculations with the human virus were made intranasally in most instances, whereas the swine virus was frequently administered by other routes.

That the sera from animals immune to the swine influenza virus exerted in certain instances some protection against the human influenza virus is evidenced by the fact that mice receiving mixtures of swine influenza immune serum and human influenza virus developed less extensive pulmonary lesions than their controls. These differences were in some instances so slight as to be of doubtful significance, but in other cases there was undoubted partial protection. The degree of cross-protection could not be positively correlated with the number of exposures of the serum donor to swine influenza virus, although in the only instance in which cross-neutralization by swine influenza immune serum was complete, the mice furnishing the serum had undergone repeated inoculations with swine influenza virus. The results show that sera from animals subjected to repeated inoculations with swine influenza virus are frequently capable of partially neutralizing the P. R. 8 strain of human influenza virus in the amounts employed. By decreasing the amount of virus in the mixtures a dilution might have been reached at which such sera would afford complete protection. It seemed best, however, to have each mixture contain an amount of virus sufficient to kill all or most of the control mice, in order to simplify interpretation of the results obtained.

The sera of ferrets or swine merely convalescent from infection with the human influenza virus exerted little, if any, protection against the swine influenza virus. Mice inoculated with mixtures of such sera and swine influenza virus usually died, just as did their controls. The few that survived the 6 day period of observation exhibited extensive

TABLE I

Neutralization of Swine and Human Influenza Virus with Serum from Convalescent or Immunized Animals

Animal No.		Animals from which serum was obtained	No. and route of inoculations	Serum tested against							
				Swine influenza virus, strain 15				Human influenza virus, strain P.R. 8			
				Extent of pulmonary lesions in Mouse No.				Extent of pulmonary lesions in Mouse No.			
				I	II	III	IV	I	II	III	IV
		(*a*) *Animals previously vaccinated or infected with swine influenza virus*									
Mice	Lot A	Vaccinated and immune to swine influenza virus	2–IP, 1–IN	0	0	0	0	4	4	4	4
	" B	Convalescent from swine influenza virus infection	1–IN	0	0	0	0	4	4	4	4
	" C	Vaccinated and actively immune to swine influenza virus	1–SC, 1–IN	0	0	0	0	2	3	2	3
	" D	" " " " " " " "	3–IP or SC, 1–IN	0	0	0	0	3	3	4	4
	" E	" " " " " " " "	3–SC, 1–IN	0	0	0	0	4	4	4	4
	" F	" " " " " " " "	3–IP, 1–IN	0	0	0	0	4	4	4	3
	" G	" " " " " " " "	3–SC, 1–IN	0	0	0	0	3	3	2	2
	" H	" " " " " " " "	3–IP, 1–IN	0	0	0	0	1	3	1	2
	" I	" " " " " " " "	3–SC, 1–IN	0	0	0	0	4	3	4	—
	" J	" " " " " " " "	3–IP, 1–IN	0	0	0	0	3	3	2	2
	" K	" " " " " " " "	3–SC, 1–IN	0	0	0	0	4	4	3	3
	" L	" " " " " " " "	3–IP, 1–IN	0	0	0	0	3	3	2	2
	" M	" " " " " " " "	1–SC, 1–IN	0	0	0	0	1	1	2	2
	" N	" " " " " " " "	1–IP, 1–IN	0	0	0	0	2	2	1	2
	" O	" " " " " " " "	2–IP, 1–IN	0	0	0	0	0	0	0	0
Ferrets	5-5, 5-7, 5-8, 6-0	" " " " " " " "	1 or 2 SC, 1–IN	0	0	0	0	4	4	4	—

Swine	1931–32	Pooled sera from hogs convalescent from swine influenza*	1 or more IN	0	0	0	—	4	4	2	2
	15-30	Vaccinated against swine influenza	2–IM	0	0	0	0	4	4	4	—
	15-61	" " " "	2–IM	0	0	0	1	4	4	3	—
	10-77	Convalescent from infection with swine influenza virus alone	1–IN	0	0	0	0	4	4	4	—
	13-56	Convalescent from swine influenza	1–IN	0	0	0	0	4	3	4	4
	11-47	Vaccinated and immune to swine influenza	3–IM, 1–IN	0	0	0	0	3	4	3	4
	14-44	Convalescent from swine influenza	2–IN	0	0	0	0	2	3	3	3
Rabbits	13-19	Vaccinated with swine influenza virus	2–IP	0	0	0	0	4	4	3	4
	13-20	" " " " "	2–IP	0	0	0	0	4	4	4	4
Horse	I.H. 4	Hyperimmunized against swine influenza virus (Laidlaw *et al.*)	6–SC	0	0	0	0	3	2	2	3
		(*b*) *Animals previously vaccinated or infected with human influenza virus*									
Mice	Lot 4	Vaccinated and immune to P.R.8 human influenza virus	1–SC, 1–IP, 1–IN	4	4	3	2	0	0	0	0
	" 1	" " " " " " " "	1–SC, 1–IP, 3–IN	0	0	0	0	0	0	0	0
	" 2	" " " " Phila. " " "	1–SC, 1–IP, 2–IN	0	0	0	0	0	0	0	0
	" 3	" " " " " " " "	2–IP, 3–IN	0	1	0	1	0	0	0	0

IP = intraperitoneally.
IN = intranasally.
SC = subcutaneously.
IM = intramuscularly.
0 = mouse with no detectable influenzal lung lesions postmortem.
1 = mouse with influenzal pneumonia involving up to ¼ of lung at postmortem.
2 = " " " " " from ¼ to ½ " " "
3 = " " " " " " ½ " ¾ " " "
4 = " " " " " " ¾ to all of lung at postmortem. Mice showing lung lesions to this extent usually died.

* The term swine influenza indicates that both swine influenza virus and *H. influenzae suis* were used for infection.

TABLE I—*Concluded*

Animal No.		Animals from which serum was obtained	No. and route of inoculations	Serum tested against							
				Swine influenza virus, strain 15				Human influenza virus, strain P. R. 8			
				Extent of pulmonary lesions in Mouse No.				Extent of pulmonary lesions in Mouse No.			
				I	II	III	IV	I	II	III	IV
		(*b*) *Animals previously vaccinated or infected with human influenza virus*									
Ferrets	3-00	Alaska strain human influenza virus	1–IN	4	4	3	3	0	0	0	0
	3-26	" " " " "	2–IN	4	4	4	4	0	0	0	0
	"	" " " " "	2–IN, 1–SC	4	4	4	4	0	0	0	0
	2-29	Phila. " " " "	1–IN	4	4	4	4	0	0	0	0
	2-78	" " " " "	2–IN	4	4	4	4	0	0	0	0
	3-12	Alaska " " " "	1–IN	4	4	4	—	0	0	0	0
	"	" " " " "	4–IN, 1–SC	4	2	1	3	0	0	0	0
	3-25	" " " " "	2–IN	4	4	4	4	0	0	0	0
	"	" " " " "	3–IN, 1–SC	4	2	3	3	0	0	0	0
	2-15	Phila. " " " "	3–IN	2	1	1	1	0	0	0	0
	1-44	P.R.8 " " " "	3–IN, 1–SC	0	0	0	0	0	0	0	0
	1-41	" " " " "	4–IN	0	1	1	—	0	0	0	0
	1-76	" " " " "	4–IN	0	0	0	0	0	0	0	0
	1-14	" " " " "	5–IN, 1–SC	0	0	2	0	0	0	0	0
Swine	16-18	Exposure to swine infected with P.R.8 strain human influenza virus	Contact infection	4	4	4	4	0	1	1	0
	16-15	Convalescent from human influenza virus infection, P.R.8 strain	1–IN	4	4	4	4	0	0	0	0
	15-99	Same as above	1–IN	4	4	4	3	0	0	0	0
	16-05	" " "	1–IN	4	4	4	3	0	0	0	0
Rabbit	1-29	Vaccinated with human influenza virus P.R.8	2–IP	0	0	0	0	0	0	0	0
Horse	I.H. 2	Hyperimmunized against W.S. strain human influenza virus (Laidlaw *et al.*)	20–SC	0	0	1	0	0	0	0	0

		(c) Animals previously infected with both swine and human influenza virus									
Swine	16-45	Normal		4	4	4	4	4	4	4	4
	"	Convalescent from P.R.8 virus infection (19 days)	1–IN	4	4	4	1	0	0	0	1
	"	31 days after P.R.8 virus IN, 11 days after swine influenza virus	1–IN	0	0	0	0	0	0	0	0
	16-57	Normal		4	4	4	4	4	4	4	4
	"	Convalescent from P.R.8 virus infection (19 days)	1–IN	4	4	4	4	0	0	0	0
	"	11 days after 2nd P.R.8 virus inoculation, 31 days after P.R.8 virus	2–IN	4	4	4	4	0	0	0	0
	"	11 days after swine influenza virus	1–IN	0	0	0	0	0	0	0	0
	16-59	Normal		4	4	4	4	4	4	4	4
	"	Convalescent from P.R.8 virus infection (19 days)	1–IN	4	4	4	—	0	0	0	0
	"	19 days after 2nd P.R.8 virus inoculation	2–IN	3	4	4	4	0	0	0	0
	"	After inoculation of swine influenza virus	1–IN	0	0	0	0	0	0	0	0
		(d) Control animals									
Mice		Normal		4	4	4	4	4	4	4	4
Ferret		"		4	4	4	4	4	4	4	4
Swine		"		4	4	4	4	4	4	4	4
Rabbit		"		4	4	4	4	4	4	4	4
Horse		"		4	4	4	4	4	4	4	4

influenzal pulmonary lesions at autopsy. However, the sera from ferrets and mice that had undergone repeated exposures to human influenza virus tended to neutralize the swine influenza virus and the degree of this heterologous neutralization corresponded roughly with the number of virus exposures undergone by the serum donor. It appeared from this that, while the virus-neutralizing properties of sera of animals convalescent from human influenza virus infection were specific, hyperimmunization tended to broaden the range of activity of these sera so that they finally acquired the ability to neutralize the heterologous as well as the homologous virus. Thus the sera of the repeatedly inoculated ferrets, 1-44 and 1-76, completely neutralized an amount of swine influenza virus that proved fatal for all the control mice, while sera from ferrets 2-29 and 3-00, receiving only a single inoculation of human influenza virus, were devoid of protective power against the same amounts of swine influenza virus. Furthermore, in the case of ferrets 3-12 and 3-25, the neutralizing capacity of the serum against swine influenza virus increased during the course of successive reinoculations with human influenza virus. The serum of mice (lots 1, 2, 3) vaccinated with human influenza virus and subjected thereafter to repeated intranasal inoculations with the human virus, also protected completely against the swine virus. Similarly, the sera of animals of two non-susceptible species (rabbit 1-29 and horse I. H. 2), immunized against the human influenza virus, neutralized the heterologous virus, whereas the serum of animals of the same species (rabbits 13-19–20 and horse I. H. 4), immunized against swine influenza virus, protected but little against the human influenza virus.

The specificity of convalescent serum for the homologous virus is well demonstrated in the case of swine 16-45, 16-57, and 16-59, which, as a result of a primary infection with human influenza virus, developed antibodies effective only against the human strain. When, subsequently, the swine influenza virus was used for reinoculation of the animals, a specific antibody response to swine virus occurred and the serum then neutralized both the human and swine strains of influenza virus. Repeated inoculation of ferrets or mice with human influenza virus does, however, result in the formation of antibodies effective against the swine influenza virus. It should be noted in this connection that ferrets receiving multiple intranasal inoculations with virus exhibited evidence of illness only following the initial inoculation.

DISCUSSION

The findings presented indicate that certain antigenic components are possessed in common by the human and swine influenza viruses. They also suggest that the common antigen is present in a more active concentration in the human than in the swine virus.

It seems likely that the virus neutralization test is of sufficient accuracy to indicate the nature of the virus involved in earlier influenzal infections of man or animals. The tendency of repeated exposures to virus to diminish the specificity of the reaction would constitute the main source of error. Complete neutralization of both human and swine influenza virus by an unknown sample of serum might mean that its donor had undergone earlier infections with both viruses, or that he had suffered repeated exposures to one or the other virus. Complete neutralization of one virus with no neutralization, or only partial neutralization, of the other would probably indicate, however, the character of the earlier infection.

SUMMARY

Human and swine influenza viruses were regularly neutralized by their homologous immune sera. However, the sera of animals convalescent from infection with either the swine or human influenza virus possessed little, if any, neutralizing capacity for the heterologous virus. Hyperimmunization of animals against swine influenza virus tended to increase the neutralizing capacity of their sera for human influenza virus, but in an inconstant fashion, whereas repeated inoculations with human influenza virus frequently resulted in sera with strong neutralizing activities against swine influenza virus. These observations serve to emphasize both the immunological distinctiveness and the interrelationships of swine and human influenza viruses.

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