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Significance of Antigenic Differences Among Strains of Influenza A Virus in Reinfection of Ferrets.*

JOHN Y. SUGG AND T. P. MAGILL.

From the Department of Bacteriology and Immunology, Cornell University Medical College, New York, N.Y.

The present paper presents data concerning the significance of the antigenic differences among strains of influenza A virus in the reinfection of ferrets. It has been commonly accepted[1-3] that following an influenza virus infection those animals are, for a time thereafter, "solidly immune" to reinfection with that same strain. However, there are few data showing whether or not during the period of "solid immunity" to one strain, ferrets would fail to show clinical manifestations when reinoculated with strains of virus antigenically related to but different from the strain used for the original infection.

Materials and Methods. Ferrets were inoculated intranasally either with influenza A virus or with control material. A febrile reaction following inoculation was taken as the index of clinical infection. Pools of infected allantoic fluid served as the source of virus and uninfected allantoic fluid was used for the control material. The pools of allantoic fluid were collected before the start of the study and were preserved in the CO_2 ice chest until immediately before use. The ferrets were anesthetized by intraperitoneal injections of nembutal. While anesthetized, the animals were bled from the heart and then were given 1.5 cc of inoculum intranasally. In addition to the bleedings made at the time of inoculation, blood was obtained from each animal during the period of convalescence. The virus-neutralizing antibody content of each serum was determined by means of the mouse protection test; the titers are expressed in terms of the initial dilution of serum which protected 50% of the mice from death.[4] All serums showing protection when diluted 1-1024 or more are recorded as having an antibody titer of 2^{10}.

Five groups of 3 ferrets each were used. Three groups (9 animals) were infected with the CC[5] strain of influenza A virus; the 6 remaining animals which were to serve as controls, were inoculated with uninfected allantoic fluid. Six weeks later all of the animals were reinoculated: 3 infected and 3 control animals received the CC strain of virus, 3 infected and 3 controls received the antigenically related WS[1] strain, and 3 infected animals re-

* From the Strain Study Center, Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army.

1 Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2**, 66.

2 Francis, T., Jr., and Stuart-Harris, C. H., *J. Exp. Med.*, 1938, **68**, 813.

3 Francis, T., Jr., *J. Exp. Med.*, 1939, **69**, 283.

4 Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

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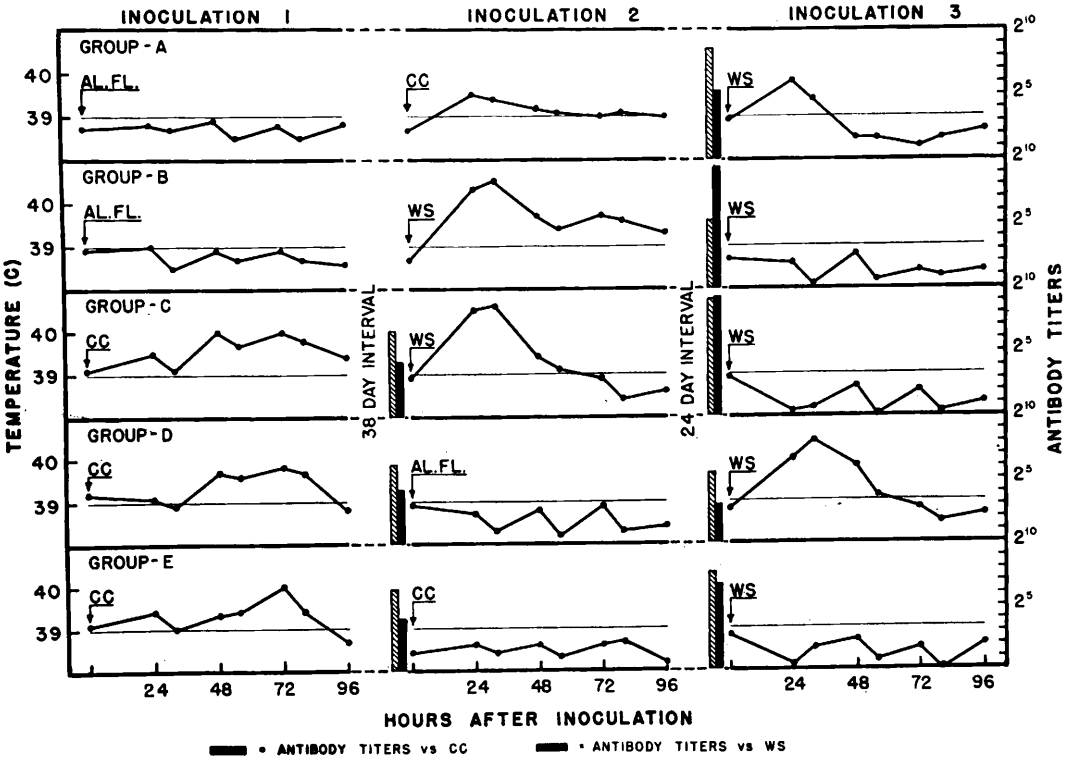
CHART 1.

Group data. Antibody titers at the time of, and temperature courses following, reinoculation of different strains of influenza virus.



ceived uninfected allantoic fluid. Four weeks after the second inoculation all of the animals were inoculated intranasally with the WS strain.

Results. Temperatures obtained during the 4-day periods following each inoculation, together with antibody titers of the serums obtained at various intervals of time are presented in Chart 1 and in Table I. The results of the neutralization tests with the serums obtained at the time of the first inoculation are omitted because none of those serums possessed demonstrable antibodies against either the CC or the WS strains.

Chart 1 shows graphically, for each of the 5 groups, the average antibody titer at the time of inoculation and the average temperature course following inoculation. Each temperature point and each antibody titer represents the average of data obtained from 3 animals except in the case of Group A, inoculation 3; one of the animals of that group developed an ear infection following inoculation 2 and was discarded.

It is evident (Chart 1) that 4 to 10 weeks following the initial infection ferrets were susceptible to clinical reinfection with influenza virus. The susceptibility to reinfection was influenced by the strain of virus used for reinoculation and by the previous antigenic experience of the animals. Inoculation 2 shows the influence of the strain of virus used for reinoculation upon the febrile responses of ferrets which had had the same previous antigenic experiences. Groups C, D and E all had been initially infected with CC virus. When retested 6 weeks later, the Group C animals, which were inoculated with the antigenically related but different WS strain, gave a pronounced febrile response. On the other hand, the Group E animals, which were retested with CC, showed no more evidence of clinical infection than did the Group D animals which received uninfected allantoic

TABLE I.

Individual Data, Antibody Titers and Febrile Responses to Reinoculation of Different Strains of Influenza Virus.

		Inoculation 1						Inoculation 2						Inoculation 3			
				Antibody titer 2 wks after inoc.		Antibody titer at time of inoc.				Antibody titer 2 wks after inoc.		Antibody titer at time of inoc.				Antibody titer 2 wks after inoc.	
Group	Ferret No.	Inoculum	Febrile response	CC	WS	CC	WS	Inoculum	Febrile response	CC	WS	CC	WS	Inoculum	Febrile response	CC	WS
A	1	Al. Fl.	—	0	0	0	0	CC	+	x*	x	x	x	x	x	x	x
	2	”	—	0	0	0	0	”	+	28.8	24.5	28.8	25.3	WS	+	28.5	27.7
	3	”	—	0	0	0	0	”	+	29.5	24.7	28.5	25.3	”	+	28.5	28.5
B	4	”	—	0	0	0	0	WS	+	26.5	210	25.5	29.5	”	—	26.5	29.8
	5	”	—	0	0	0	0	”	+	25.5	210	25.5	29.3	”	—	25.5	29.3
	6	”	—	0	0	0	0	”	+	25.5	29.5	24.5	29.5	”	—	25.5	210
C	7	CC	+	28.5	22.6	26.5	23.3	”	+	29.5	29.5	28.5	28.3	”	—	28.5	29.3
	8	”	+	28.5	24.3	27.2	24.7	”	+	210	210	29.5	210	”	—	29.8	210
	9	”	+	28.3	24.3	26.5	24.5	”	+	29.5	26.2	29.3	29.3	”	—	29.5	29.3
D	10	”	+	28.5	22.8	27.2	25.3	Al. Fl.	—	26.5	24.3	26.5	24.3	”	+	29.8	210
	11	”	+	28.8	23.5	26.3	23.3	”	—	25.6	22.3	25.3	21.6	”	+	29.5	29.5
	12	”	+	28.5	23.3	24.5	23.3	”	—	24.5	22.6	23.7	21.5	”	+	29.5	210
E	13	”	+	28.5	24.3	26.5	23.3	CC	—	28.5	27.2	26.7	25.5	”	—	28.5	29.3
	14	”	+	28.5	25.3	26.5	24.3	”	—	28.5	26.5	27.2	25.8	”	—	28.8	29.3
	15	”	+	28.5	22.8	26.5	24.5	”	—	28.5	27.5	28.5	27.8	”	—	28.5	28.5

* Ferret No. 1 developed an ear infection following inoculation 2 and was discarded.

fluid. Inoculation 3 shows the influence of the previous antigenic experience upon the responses of ferrets which were inoculated with the same amount of the same virus suspension. Groups A and D, whose previous influenza virus experience consisted of one inoculation with CC (respectively, 4 and 10 weeks previously), had definite febrile responses following inoculation of WS virus. In contrast, the Group E animals whose previous influenza virus experience likewise had been limited to contact with CC but who had received 2 inoculations of that virus (4 and 10 weeks previously) proved to be clinically immune, as did Groups B and C, which had undergone previous infection with WS.

The influence of the strain of virus used for inoculation, and also the influence of the previous antigenic experience were associated with the height of titer of strain-specific antibodies. Comparison of the antibody titers at the time of inoculation with the subsequent temperature courses shows that all groups which gave evidence of clinical infections when retested (Group C, inoculation 2; and Groups A and D, inoculation 3) had lower average titers of antibodies reactive with the strain used for the reinoculation than did any of the groups found to be immune to clinical reinfection (Group E, inoculation 2; and Groups B, C and E, inoculation 3). The lowest titer of the clinically immune groups occurred in Group E, inoculation 3, and was slightly less than 2^7; the highest titer of the clinically susceptible groups occurred in Group A, inoculation 3, and was slightly more than 2^5. Thus, the group data indicate that there was a critical antibody level which separated a state of relative susceptibility from a state of relative resistance, and that the strain-specific titer at the time of inoculation can be taken as an index of that critical antibody level. However, that titer is not necessarily an index of the total amount of antibodies which will be available to combat clinical infection because the speed with which the animal can produce additional antibodies must also be taken into consideration. That the speed of response is influenced by the extent of previous experience of the animal with influenza virus antigens is indicated by the data of the individual animals (Table I).

Table I shows that Ferrets No. 13 and 14 (Group E), at the time of inoculation 3, had WS antibody titers of $2^{5.5}$ and $2^{5.8}$ but showed no signs of infection following inoculation. In contrast, Ferrets No. 2 and 3 (Group A) both had titers of $2^{5.3}$ against WS yet they gave a mild febrile response following inoculation of that strain of virus. The difference between a titer of $2^{5.3}$ on the one hand and of $2^{5.5}$ or $2^{5.8}$ on the other hand probably is insignificant and hardly can explain the difference in the responses of those animals to inoculation of the WS strain of virus. There was, however, a significant difference in the previous influenza virus experience of these ferrets. The more resistant animals (No. 13 and 14) had had the more extensive experience in that they had had 2 inoculations of the CC strain whereas Ferrets No. 2 and 3 had had only one. That the additional experience conditioned the immunological mechanism to respond more quickly is suggested by the fact that 2 weeks subsequent to inoculation Ferrets No. 13 and 14 had significantly higher WS antibody titers than did Ferrets No. 2 and 3. A sufficiently quick antibody response obviously would have enabled the animals to render the virus ineffective before it had multiplied enough to produce symptoms of clinical infection, irrespective of the antibody titer at the time of inoculation.

Discussion. The present investigation dealt with the resistance of ferrets to clinical reinfection with antigenically related but different strains of influenza virus. The main point was that 4 to 10 weeks following an initial infection the animals, in all cases, were immune to reinfection with the same strain, but were susceptible to reinfection with a strain of virus which was antigenically related to but different from the strain used for the original infection. It would seem, therefore, that the antigenic differences which are known to exist among the A strains of influenza virus are of sufficient magnitude to be of practical importance in problems dealing with immunity to influenza. For example, an immunization procedure might be adequate to produce a high degree of resistance to infection with some strains of influenza virus but afford little protection against infection with

other, although antigenically related strains.

The results indicate that for a constant amount of virus there was a critical level of specific antibodies which separated a state of relative resistance from a state of relative susceptibility. However, the height of titer at the time of inoculation was not always an index of the resistance of the animal to clinical infection. When ferrets which had approximately the same titers of specific antibodies were inoculated with the same amount of the same virus suspension, the animals which had had the more extensive previous experience with influenza virus strains were likewise the more resistant to clinical infection. The more extensive experience apparently endowed those animals with a more active immunological mechanism which enabled them to mobilize more quickly a higher concentration of strain-specific antibodies. In so far as protection against clinical infection is concerned the protective effect of antibodies is not limited to the quantity present at the time of inoculation but is dependent upon the amount made available before the infecting agent has multiplied sufficiently to evoke clinical manifestations.

Summary. Four to 10 weeks following an initial infection with influenza A virus, ferrets were found to be immune to clinical reinfection with that same strain of virus but were susceptible to reinfection with a strain of virus which was antigenically related to but different from the strain used for the original infection.

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Filamentous Forms of Newcastle Virus.

F. B. BANG. (Introduced by Carl TenBroeck.)

From the Rockefeller Institute for Medical Research, Princeton, N.J.

Relatively few of the animal viruses have been purified and studied under the electron microscope.[1] This preliminary report deals with the causative agent of Newcastle disease of chickens.

Four strains of virus have been studied, 2 isolated in New Jersey, 2 in California. Strain B was isolated from a field case by Dr. F. R. Beaudette and furnished to us in the 1st embryo passage. Strain W was isolated by us from an outbreak of the disease in a flock of White Leghorns at Bound Brook, N. J. Strain Nap is a laboratory strain isolated by Dr. J. R. Beach in California. These 3 strains are identified as Newcastle virus by their behavior in the developing chick embryo, which they kill with specific hemorrhagic lesions of the brain, feather follicles, and entire embryo in 48 to 84 hours,[2] by their ability to agglutinate chicken red blood cells, by the neutralization of these effects by specific antisera against known strains, and by the reproduction of the natural disease in chickens.[3,4] Strain Cg179, also furnished by Dr. Beach, is highly virulent for chickens and is specifically neutralized by antisera in the embryo.

All four of these strains when partially purified from allantoic fluid by 2 cycles of differential ultracentrifugation show filamentous forms which predominate on the screen of the electron microscope. (Fig. 1 and 2); the phagelike structure is not a constant feature of our preparations, but at no time have we failed to demonstrate filamentous forms. That these forms represent active virus is indicated by their presence in concentrates of the allantoic fluid as early as 24 hours after inoculation until 48 to 72 hours when death of the embryo occurs; by their presence at tem-

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