NEUTRALIZING ANTIBODIES IN HUMAN SERUM AFTER INFLUENZA A

The Lack of Strain Specificity in the Immunological Response

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Magill and Francis (1), by means of neutralization tests with specific immune animal serum, demonstrated that there were antigenic differences between certain strains of influenza A virus (2). This discovery was confirmed and considerably extended in subsequent investigations by these authors (3, 4) and by Smith and Andrewes (5). Magill and Sugg (6) reported that with human serum the titer of neutralizing antibodies may be dependent upon the virus strain used in the test. They also reported that during convalescence from influenza A neutralizing antibodies against several antigenically different strains of the virus were increased though not always to the same extent. Taylor and Dreguss (7) obtained essentially similar results. Andrewes, Smith, and Stuart-Harris (8) presented evidence which indicated that influenza A might also result in the production of antibodies against swine influenza virus. This finding was confirmed by Hare and Riehm (9).

The fact that there are quantitative, though not qualitative, antigenic differences between strains of influenza A virus would seriously complicate the study of immunity to influenza A if it were found that human beings produced a relatively strain-specific antibody response to infection by a particular virus strain. On the other hand, if, as seems probable from the results obtained by other investigators, there is a broad immunological response to influenza A in human beings, the importance to man of antigenic differences between strains of the virus diminishes in proportion to the breadth of the antibody response and its persistence in time.

It is the purpose of this paper to present evidence which indicates that irrespective of the time which elapses after the onset of the disease, there is an almost complete lack of strain specificity in the immunological response which follows influenza A.

Materials and Methods

Sera.—Serum specimens were obtained from five adult patients who were confined in one ward of a state institution and who were ill with influenza A at approximately

the same time during February, 1939. The epidemic affecting this institution has been described previously (10). Specimens of serum were taken during the acute phase of illness from 2 to 4 days after the onset. Additional serum specimens were obtained approximately 10, 18, and 25 days, as well as 2, 3, 8, and 12 months, following the beginning of illness.

Viruses.—Three antigenically different strains of influenza A virus were used:

(*a*) Strain 399 was isolated from the nasopharyngeal washings of one of the patients in this series (Case No. 1). After 9 serial passages in ferrets this strain was established in mice, in which species it was carried through 15 serial passages. Two suspensions of mouse lungs infected with this strain were employed. The 50 per cent mortality end points of these two suspensions were $10^{-4.5}$ and $10^{-5.4}$, respectively.

(*b*) The PR8 strain (11) after preliminary ferret passages had been carried through 332 serial passages in mice. The 50 per cent mortality end point of the suspension used was $10^{-6.5}$.

(*c*) The W. S. strain (12) was obtained through the courtesy of Dr. C. H. Andrewes. Exact data as to the number of animal passages are not available. The 50 per cent mortality end point of the suspension used was $10^{-6.9}$.

One strain of swine influenza virus (No. 1976), which was obtained through the courtesy of Dr. R. E. Shope, was used. The suspension had a 50 per cent mortality end point of $10^{-3.7}$.

Standard suspensions of mouse lungs infected with each of the viruses mentioned above were prepared as described previously (13) and were stored in a low temperature cabinet (14) at $-76°C$.

Neutralization Tests.—Neutralization tests with each of the virus strains indicated above were carried out on the various serum specimens from each of the five cases of influenza A. The technique of the neutralization test has been described previously (10). A constant quantity of the desired strain of virus was mixed with serial four-fold dilutions of serum. Serum dilution end points and virus titration end points were calculated by the 50 per cent mortality method of Reed and Muench (15).

Neutralizing Capacity.—The neutralizing capacity of each serum against each strain of virus was calculated from the results of the neutralization tests by means of the equation

$$\log b = \log y - (a \cdot \log x) \tag{16}$$

EXPERIMENTAL

The neutralizing capacities of multiple serum specimens obtained from five cases of influenza A were determined against three antigenically different strains of influenza A virus and one strain of swine influenza virus. The results are presented in Table I. It will be seen that in the serum of Case 1, from whose nasopharyngeal washings Strain 399 was recovered, there occurred an increase in neutralizing capacity of more than log 2.60 against the homologous strain of virus following infection. Closely similar increases in antibody level were also observed with both the PR8 and W.S. strains of influenza A virus, as well as with swine influenza virus.

It will also be noted that, with the single exception of Case 2, all five cases

TABLE I

The Neutralizing Capacity of Human Serum against Antigenically Different Strains of Influenza A Virus and Swine Influenza Virus at Intervals Following Influenza A

Case No.	Days after onset of illness	Neutralizing capacity of serum against different viruses			
		Influenza A virus			Swine influenza virus
		PR 8	W.S.	399	
		log	*log*	*log*	*log*
1	3	3.93 or <	4.12 or <	2.36 or <	3.19
(Source of	11	6.53	5.99	4.96	4.93
strain 399)	18	6.75	5.99	4.96	4.78
	25	6.53	6.29	4.96	4.93
	60	5.67	5.99	4.53	—
	90	5.05	5.70	4.53	3.92
	240	4.94	4.99	3.67	4.32
	360	4.62	4.81	—	3.48
2	3	5.80	4.00	1.83 or <	5.97
	11	7.62	4.57	4.27	7.47
	18	7.40	4.57	4.27	6.99
	25	7.62	4.43	3.56	6.56
	60	6.53	4.00	3.41	6.56
	90	6.10	4.43	3.23	6.13
	240	5.67	4.57	2.70	5.83
	360	5.74	4.43	2.70	6.13
3	2	5.67	5.86	4.05 or <	5.83
	10	7.62	7.44	6.65	7.43
	17	7.40	7.59	6.65	7.28
	24	7.40	7.44	6.65	7.28
	59	6.53	6.95	5.93	6.31
	89	6.53	6.95	5.35	7.28
	239	6.23	6.29	>5.78	6.56
	360	5.80	6.72	4.92	6.40
4	2	4.62	4.12 or <	3.18 or <	4.83
	10	6.96	5.99	4.92	6.13
	17	6.53	5.86	4.92	6.13
	24	5.67	4.99	4.92	6.40
	59	5.51	4.99	4.92	5.54
	89	4.80	4.99	4.05	5.70
	239	4.80	4.81	4.05	5.54
	—	—	—	—	—
5	4	5.96	6.72	4.92	5.26
	12	7.70	7.74	6.21	6.71
	19	7.70	7.84	5.93	7.28
	26	8.12	7.32	—	—
	61	6.53	7.15	5.93	6.30
	241	5.80	6.72	4.92	6.13
	360	5.67	6.72	4.92	5.54
Mean	2–4	5.20	4.96	3.27	5.02
all	10–12	7.29	6.35	5.40	6.53
cases	17–19	7.16	6.37	5.35	6.59
	24–26	7.07	6.09	5.02	6.29
	59–61	6.15	5.81	4.94	6.18
	89–90	5.62	5.52	4.29	5.76
	239–241	5.49	5.48	4.23	5.68
	360	5.45	5.67	4.18	5.39

were found to have produced almost identical quantities of neutralizing antibodies against each of the virus strains used. Although in the sera from Case 2 similar concentrations of antibodies were found against Strain 399 and the PR8 strain of influenza A virus, as well as swine influenza virus, only a slight increase in antibodies was demonstrable against the W.S. strain.

The acute phase sera of the five patients varied considerably in their capacities to neutralize the different virus strains. Nevertheless, the acute

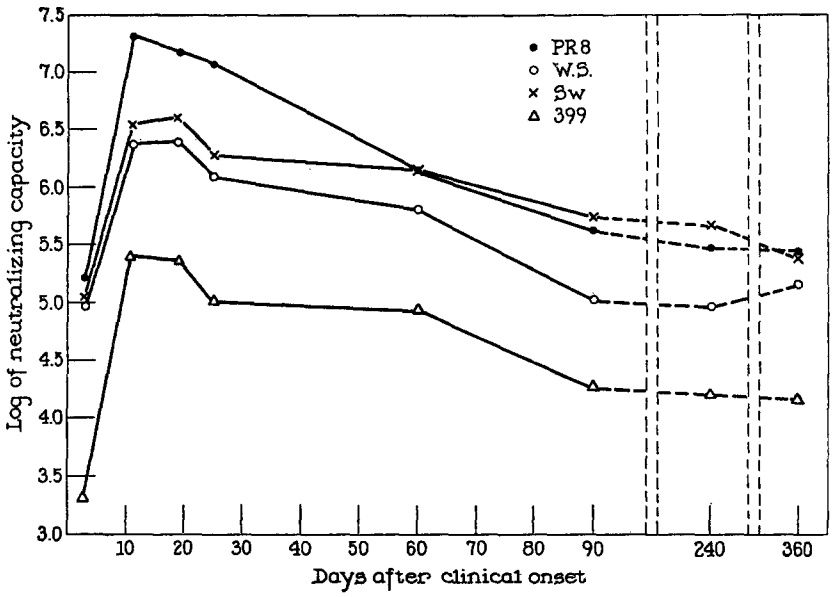

FIG. 1. Mean neutralizing capacities against three antigenically different strains of influenza A virus and swine influenza virus of serum obtained from five cases of influenza A at intervals after onset.

phase serum from each individual had a relatively constant neutralizing capacity against the PR8 and W.S. strains of influenza A virus and swine influenza virus. In the acute phase sera of all cases, however, the neutralizing capacities against Strain 399 were definitely lower than against the three other viruses.

In Fig. 1 the mean neutralizing capacities of the sera of the five cases against each of the four viruses are shown graphically. The mean neutralizing capacities have been plotted against the time after clinical onset at which the sera were obtained, and the experimental points have been connected by straight lines. It seems apparent that in these five cases of influenza A the observed increases in neutralizing antibody levels which followed infection

were as readily demonstrable when one strain of influenza A virus was used as when another antigenically different strain was employed. Furthermore, closely similar alterations in antibody levels were also encountered even when the antigenically distant swine influenza virus was used in the neutralization tests. It will be observed that the shape of the mean antibody level time curves shown in Fig. 1 were almost identical irrespective of the strain of influenza A virus against which the sera were tested, and that even in the case of swine influenza virus, similar alterations in neutralizing antibodies with time were found.

Not only were the shapes of the antibody level curves similar, but under the conditions of these experiments it was found that the mean concentrations of neutralizing antibodies, at any interval studied, against the PR8 or W.S. strains of influenza A virus or even swine influenza virus were not very different. However, the mean antibody levels found when Strain 399 of influenza A virus was used in neutralization tests were at each interval definitely lower than those obtained with the other virus strains. It will be recalled that Strain 399 was recovered from the nasopharyngeal washings of Case 1. This strain of virus was not only causally related to the illness observed in Case 1 but undoubtedly was either antigenically very similar, if not identical, to those strains of virus which were associated etiologically with the disease encountered in the other four cases under study. It should be pointed out that Strain 399 had been carried through only a relatively small number of passages in mice and, therefore, it may not have acquired full virulence for this species. Under these circumstances the 50 per cent mortality titration end point may not have given a fair indication of the quantity of virus present in the suspension. If a greater number of virus particles actually were contained in the suspension than might have been expected from the results of the titrations, it seems obvious that the determined neutralizing capacity of a serum would *apparently* be lower than otherwise.

It has been shown previously (17) that the increased antibody levels which follow influenza A rapidly decline. The results shown in Fig. 1 present additional evidence in this regard. It will be seen that even between the 11th and the 25th day after onset of the disease there was with each of the strains of virus used an indication that a slight decrease in antibody concentration had occurred. The progressive lowering of neutralizing capacities continued during the 2nd month after onset and was somewhat more rapid during the 3rd month. Between the 3rd and the 12th months relatively little additional decrease in antibody level occurred. Three months after onset the mean neutralizing capacity against the four virus strains of the serum of the five cases was only log 0.65 higher than during the first days of their illness, and at 12 months it was but log 0.45 greater than in the acute phase of the disease.

DISCUSSION

Quantitative antigenic differences between strains of influenza A virus are readily demonstrable in experiments with laboratory animals if tests are carried out shortly after primary immunization (3) or following initial infection (5). However, when longer intervals are allowed to elapse (3), or hyperimmunization is carried out (4), or repeated infections are produced (5, 8), antigenic differences between strains of the virus become less definite or may even be undemonstrable. This is usually taken to indicate that laboratory strains of influenza A virus possess a number of antigenic components any of which may be present in varying concentration but many or most of which are present in some concentration in all strains studied.

Owing to the fact that the majority of normal human beings, unlike normal laboratory animals, possess in their serum considerable quantities of neutralizing antibodies against influenza A virus (18), no matter which strain of the virus is used in the test, it seems reasonable to assume that these antibodies are one expression of previous contact with the virus. Furthermore, it seems probable that by the time adult life is reached the greater proportion of human beings may have had a number of opportunities for contact with the virus and that in most instances some of these contacts would have resulted in one or more clinical or subclinical infections by the agent. Under these circumstances an attack of influenza A during adult life would be due usually to reinfection by the virus and should result in an immunological response similar to that observed in hyperimmunized or repeatedly infected laboratory animals.

The results of the experiments reported herein indicate that there is an almost complete lack of strain specificity in the neutralizing antibody response which follows influenza A in adult human beings. Furthermore, they indicate that the antibodies produced as a result of the infection possess a very broad reactivity since the concentrations demonstrable with the antigenically distant swine virus were almost identical to those demonstrable with different strains of influenza A virus itself. Finally, it appears that the very rapid and progressive decrease in antibody levels which occurs with increasing time after influenza A is not merely the result of a diminution in antibodies directed only against minor antigenic components in the virus strain responsible for the infection, since this decrease is quantitatively identical whether this strain or another is used to determine antibody concentrations.

SUMMARY

The increased concentrations of neutralizing antibodies against influenza A virus in human serum which occur after influenza A do not differentiate between antigenically different strains of this virus or swine influenza virus but instead appear to possess equal reactivity against these agents. The decrease

in antibody levels which occurs with time is also independent of the strain of virus used to measure it.

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