THE SIGNIFICANCE OF ANTIGENIC DIFFERENCES AMONG STRAINS OF THE "A GROUP" OF INFLUENZA VIRUSES

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The existence of antigenic differences among the virus agents of influenza is now a generally accepted fact. The difference between the so called "A group" and the so called "B group" is commonly taken into account, but the differences within the "groups" have in general been considered to be of little practical importance. The purpose of the present paper is to report data which show that in some instances the antigenic differences among strains belonging to the "A group" are of sufficient magnitude to warrant their being taken into consideration in all problems dealing with the immunity and epidemiology of influenza.

Methods and Materials

The investigation consisted of mouse protection tests with serums from 40 influenza patients, against 7 different strains of virus. Two strains, PR8 (1) and TM (2), were established laboratory strains, representative of "group A" and "group B." The other 5 strains CC, AN, KD, MR, and VH are of special interest because they had been isolated during the outbreak studied in the investigation; in the present paper they are referred to as the "1941 strains." These strains, although certainly not identical, do have some antigenic relationship to each other and to the PR8 and would, therefore, be included within the "A group"; their antigenic properties have been described previously (3).

The serums were from patients who had influenza during an outbreak that occurred during January and February, 1941, among the nurses of The New York Hospital. Two samples of serum were obtained from each person: the first was gotten within the first few days of the time of onset of illness; and the second, 2 to 3 weeks later.

The virus suspensions used in the tests contained, in the case of the 5 recently isolated strains and also in the case of TM, approximately 500 lethal doses for mice, per inoculated dose; in the case of PR8 the suspensions contained approximately 1000 lethal doses. The serums were tested in 2 lots. Each lot, or approximately half of the total number of pairs were tested at the same time in the same experiment against a suspension of one of the strains of virus. Each serum was tested in a series of threefold dilutions. Mixtures of 0.2 cc. of virus suspension and 0.2 cc. of serum dilution were incubated at 37°C. for 30 minutes, after which 0.05 cc. to 0.06 cc. quantities were dropped into the nostrils of groups of 3 lightly ether-anesthetized Swiss mice. The tests were terminated on the 9th day.

TABLE I

Results of Protection Tests of Serums from 40 Patients against 7 Strains of Influenza Virus

(The titres are expressed in terms of the dilution of serum which protected 50 per cent of the mice from death.)

Patient	Titres at time of onset of illness							Titres 2 to 3 weeks after illness						
	Strain of virus							Strain of virus						
	PR8	CC	AN	KD	MR	VH	TM	PR8	CC	AN	KD	MR	VH	TM
1. J. C.	79	0	0	0	4	0	2	250	5	5	18	35	19	2
2. P. M.	19	0	0	0	0	0	2	41	12	16	54	21	110	4
3. H. J.	17	0	0	6	5	16	16	250	47	47	162	142	140	16
4. L. W.	35	5	2	2	2	16	5	250	16	15	36	98	110	5
5. B. D.	19	2	5	18	2	8	2	250	143	110	243	243	170	2
6. R. K.	21	2	2	6	4	4	2	87	47	47	162	47	142	0
7. C. C.*	10	0	5	18	2	10	0	98	63	110	243	10	125	0
8. E. J.	10	0	0	0	0	5	2	19	5	5	18	5	47	2
9. M. H.	10	0	0	2	2	0	0	250	16	16	57	47	87	0
10. J. N.	20	2	7	30	7	35	2	87	63	63	243	62	243	2
11. J. H.	19	5	15	18	5	25	5	98	48	62	162	47	125	7
12. D. K.	19	5	5	18	7	16	3	250	63	110	162	62	243	2
13. Ev. R.	37	5	15	20	16	16	0	62	47	47	162	47	62	0
14. P. E.	42	15	5	62	16	143	0	98	21	47	162	47	240	0
15. M. G.	87	9	15	54	21	41	2	250	47	47	162	62	170	2
16. G. H.	8	2	4	7	5	8	2	150	143	143	192	42	250	2
17. M. T.	8	0	5	20	5	20	2	87	15	37	160	47	110	2
18. M. L.	8	0	0	0	0	9	0	90	16	16	54	37	142	0
19. A. S.	8	0	0	0	2	16	0	87	21	16	54	47	143	0
20. J. M.	8	0	0	0	2	0	2	250	2	12	54	47	62	2
21. E. R.	8	0	0	0	2	2	2	190	12	16	18	47	110	0
22. R. B.	8	2	5	18	0	19	0	19	5	12	81	7	47	0
23. F. M.	8	0	2	6	2	12	0	30	16	15	145	21	62	0
24. L. C.	4	0	0	20	0	0	0	79	47	16	160	47	79	0
25. E. G.	4	2	0	2	0	8	12	250	143	143	243	47	250	5
26. A. N.*	4	0	0	0	0	0	0	9	12	15	54	16	142	0
27. M. B.	2	0	4	19	5	5	0	87	34	110	170	142	110	0
28. M. W.	0	0	0	6	0	0	2	87	5	7	162	47	110	3
29. M. R.*	0	0	0	6	0	0	0	35	0	0	20	7	5	0
30. B. H.	0	0	0	0	0	0	0	87	5	5	4	12	5	0
31. A. R.	0	0	0	0	0	2	0	78	5	5	7	16	47	0
32. R. T.	0	0	0	0	0	0	2	39	21	16	54	16	110	3
33. A. L.	0	0	0	0	0	0	2	37	16	5	18	16	110	2
34. V. H.*	0	0	0	0	0	0	0	19	5	2	6	4	17	0
35. K. D.*	0	0	0	0	0	0	0	0	4	2	9	2	10	0
36. B. J.	0	0	0	0	0	0	0	3	0	0	0	4	5	0
37. M. G.	0	0	0	0	0	0	0	0	0	0	0	0	0	2
38. A. B.	20	12	15	36	7	81	0	30	7	15	54	12	78	0
39. B. M.	21	2	2	6	5	12	2	21	2	2	7	4	12	0
40. M. J.	41	15	12	54	12	142	2	35	12	15	54	16	142	0

* Persons from whom strains of virus were isolated.

The data are presented in Table I, in which the titres are expressed as the dilution of serum that would protect 50 per cent of the mice from death (4).

RESULTS

The data in Table I show that the calculated antibody titres, both of the "acute" and of the "convalescent" samples, were influenced by the strain of virus used to test the serums. The pronounced difference between the results obtained with the TM and with the PR8 would be expected. However, marked differences were also manifest among all of the 6 "group A" strains, which is especially significant in view of the fact that 5 of these strains had been isolated from the same localized outbreak from which the serums had been obtained. This influence of the strain of virus upon the calculated antibody titre of the serums has an important bearing upon two practical problems: the question of the rôle of circulating antibodies in the mechanism of resistance to influenza infection; and the question of the serological diagnosis of influenza. The data pertaining to these two questions will be considered separately.

Titres at the Time of Onset of Illness.—It is apparent from the data (Table I) that serums obtained at the time of onset of illness may possess quite different protective capacities against different although related strains of virus. In the case of patients 1 to 15 inclusive, the serums possessed an appreciably high (1–10 or more) protective antibody titre against the PR8, but in each instance the titre was significantly lower against one or another of the 1941 strains. For example, in case 1 (J. C.), the serum when diluted as much as 1–79 protected mice against 1000 lethal doses of the PR8 strain, but even when undiluted it failed to protect against 500 lethal doses of the CC, AN, KD, or VH strains. Also, in case 2 (P. M.), the serum protected against the PR8 when diluted 1–19 but was devoid of protection against each of the five 1941 strains.

The differences in calculated antibody titres are evident not only in comparisons of the various 1941 strains against the PR8 strain, but also in comparisons of the various 1941 strains against each other. For example, in case 3 (H. J.) the serum was effective in dilution of 1–16 against the VH strain, but had no demonstrable protective capacity against either the CC or the AN strains. In cases 10 and 17 (J. N., M. T.), the serums had titres of 1–20 to 1–35 when tested against the KD and VH strains but had little or no protective capacity against the CC strain. Similarly, in case 14 (P. E.), the serum had titres as high as 1–62 and 1–143 against the KD and VH strains but had a titre of only 1–5 against the AN strain.

The data from the 5 cases marked with an asterisk (Nos. 7, 26, 29, 34, and 35) deserve special attention because strains of virus had been isolated from those particular cases. It is important that at the time of onset of illness the serum of none of these 5 patients had any demonstrable protective antibodies against the strictly homologous strain of virus. The data in case 7 (CC) are of special

interest. The serum obtained from that patient at the time of onset of her illness had a titre of 1–18 against the KD strain and of 1–10 against both the VH and the PR8 strains, so that if judged on the basis of tests against those strains this patient would appear to be an example of the occurrence of an influenza infection in a person having a significant titre of antibodies; whereas, the tests with the homologous CC strain showed that this patient actually had no circulating antibodies reactive with the etiological agent of her infection.

Antibody Increases Evoked by the Infections.—The data in Table I show also that the detection of the antibody response is influenced by the strain of virus employed to test the serums. The differences in the ratios between the "convalescent" and the "acute" samples from the same person were often of con-

TABLE II

Examples of Significant Antibody Response Against Some Strains of Virus but Not against Other Antigenically Related Strains

Patient	Ratio between the titre of the "convalescent" sample and the titre of the "acute" sample					
	Strain of virus					
	PR8	CC	AN	KD	MR	VH
2. P. M.	41/19	12/0	16/0	54/0	21/0	110/0
8. E. J.	19/10	5/0	5/0	18/0	5/0	47/5
13. Ev. R.	62/37	47/5	47/15	162/20	47/16	62/16
26. A. N.	9/4	12/0	15/0	54/0	16/0	142/0
29. M. R.	35/0	0/0	0/0	20/6	7/0	5/0
35. K. D.	0/0	4/0	2/0	9/0	2/0	10/0
36. B. J.	3/0	0/0	0/0	0/0	4/0	5/0

siderable magnitude. However, the most interesting examples are the 7 cases in which a significant increase in the titre of the "convalescent" sample was evident in the tests against some of the strains, but not in the tests against other strains. This point can be shown conveniently by arranging the data from these 7 cases as in Table II, in the form of the ratios between the titres of the "acute" and "convalescent" samples of serum. It is evident (Table II) that, in cases 2, 8, 13, 26, and 35, little or no increase in titre was apparent in the tests against the PR8 strain, whereas very significant increases were evident in the tests against one or another (but not always all) of the five 1941 strains. On the other hand, in cases 29 and 36, significant increases were shown in the tests against the PR8 and also against the MR and the VH strains but were not demonstrable in the tests against the CC and AN strains although the latter two strains were isolated from the same outbreak as were the MR and VH strains.

It is a common practise to require for a positive diagnosis of influenza, the demonstration of an increase at least threefold in the titre of serum antibodies. When the complete data in Table I are examined on that basis, it is apparent that the number of cases which would be diagnosed as influenza would depend upon the strain of virus used to test the serums. If the PR8 strain had been used alone, only 29 of the 40 cases would have been diagnosed; if either the CC or AN strains had been used alone, only 31 would have been diagnosed as influenza. The MR strain seemed to have the broadest range of reactivity since tests with it revealed significant antibody increases in 35 of the cases. On the basis of the total tests utilizing all the strains, 36 of the 40 cases would have been diagnosed; and, in fact, these 36 cases would have been diagnosed by several combinations of two strains.

There remain however, 4 cases which showed no antibody increases against any of the 7 strains included in the tests. However, in the absence of viruses isolated from these particular cases, it is impossible to determine whether these cases represent persons who failed for some reason to give an antibody response or whether they were infected by some agent not closely related to the strains used in the tests.

DISCUSSION

The present investigation dealt with antigenic differences among strains included within the so called "A group" of influenza viruses. The existence of strain differences has been previously established (5–7); and it has been recognized that the differences frequently are reflected in serums obtained from influenza patients (8, 9). Nevertheless, the practical significance of the antigenic differences among the "A group" of strains has been open to question because it has been apparent to all who have been concerned with the problem that the differences in many instances appear to be slight, and of limited significance. The present data show, however, that in other instances the differences are of sufficient magnitude to warrant their being taken into consideration in the various aspects of the general problem of influenza.

The data show that the protective capacity of the serum against one strain of influenza virus is not necessarily a true index of the protective capacity of that serum against other, although antigenically related strains. A number of the serums obtained at the time of onset of illness possessed high protective antibody titres against some strains of virus, but not against other strains of the same group. On the basis of that evidence it seems entirely possible that at the time of onset of illness a person may possess a high serum antibody titre against some heterologous test strain of virus, but at the same time have little, if any serum protective capacity against the strictly homologous strain, even though that strain is antigenically related to the heterologous test strain. A

systematic effort was made to obtain conclusive information on that point; but in spite of numerous attempts, virus was isolated from only one person whose serum at the time of onset of infection possessed appreciably high protective antibody titres against the PR8, or other heterologous related strains included in the tests. The data in that instance are of significance, because they showed that the "acute" serum possessed appreciably high protective titres against the PR8 and against 2 of the 1941 strains, but exerted no demonstrable protective capacity against the strictly homologous strain of virus. That evidence, obviously, is insufficient to establish the point that clinical influenza occurs only in persons possessing little or no serum protective capacity against the strain of virus actually responsible for the infection. It is sufficient, however, to show that questions such as the one recently raised by Francis (10) concerning the rôle of circulating antibodies in the mechanism of resistance to influenza, can be answered only by the accumulation of evidence from experiments in which the test serums and test virus are obtained from the same patient.

The data show also that the response of protective antibodies evoked by an influenza infection may not be detected by tests of the serum against some strains of influenza virus, but can readily be detected by other antigenically related strains. That point is of practical importance from the standpoint of the serological diagnosis of influenza. In the present investigation influenza would have been diagnosed in 36 of the 40 cases by tests against several combinations of strains included within the "A group." It seems significant, however, that all 36 cases would not have been diagnosed on the basis of tests with any single one of the strains included.

SUMMARY

"Acute" and "convalescent" samples of serum from 40 patients from a localized outbreak of influenza were tested for mouse-protective antibodies against 7 different strains of influenza virus, which included 2 laboratory strains representative of "group A" and of "group B," and 5 strains from the investigated outbreak. The latter 5 strains although not identical were related to each other and to the PR8 strain. The chief point shown by the data was the considerable degree of antigenic difference among the 6 "group A" strains, evidenced by the marked differences in the protective capacities of the serums when tested against the various strains.

A number of the "acute" serums showed high protective capacity against some strains but relatively little protective capacity against other strains. In the 5 instances in which it was possible to test the "acute" serums against strictly homologous strains of virus, no protective capacity was demonstrable.

In 7 of 36 cases, the antibody responses evoked by influenza infections were not detectable by tests with some strains, but were detectable by tests against other related strains.

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