LABORATORY STUDIES ON THE INFLUENZA EPIDEMIC OF 1958

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Sometime in mid-June, 1958, an unusually large number of cases of an acute febrile disease associated with upper respiratory tract infection, headache, muscle and joint pains was reported in Pangasinan. An increasing number of cases of a similar nature was subsequently noted in other provinces and cities, including Manila. The disease which eventually reached epidemic proportions was reported as influenza, the diagnosis based solely on the clinical picture presented.

Unequivocal diagnosis of influenza is based on laboratory tests aimed at isolating the influenza virus from the respiratory tract and demonstrating a rise in antibody titer against the agent in the patient's blood. The opportunity for conducting such tests presented itself late in July when inquiry from the Directors of the University Health Service in Diliman and in Los Baños revealed that there were a number of students confined in these units with illnesses similar to what had already been described.

MATERIALS AND METHODS

Patients studied

A total of 94 patients with illnesses clinically resembling influenza were included in this study. Eighty of these were students confined at the Infirmary of the College of Agriculture in Los Baños. Five were confined at the University Health Service in Diliman. Four others were medical students, and one was a B.S. Hygiene student. The rest consisted of a visiting lecturer at the Institute of Hygiene and two patients confined at the San Lazaro Hospital.

Materials obtained

Throat washings in brain heart infusion broth were obtained from four of the patients confined at the U.P. Health Service, from two patients at the San Lazaro Hospital and from the visiting professor at the Institute of Hygiene. A total of seven throat washings were thus obtained. These specimens were collected within the first three days of illness when the patients were still febrile and acutely ill. At this time a blood sample was also collected from each of these seven patients as well as from eighty-two others from whom throat washings were not obtained. These specimens are referred to as the acute phase blood sample. Another blood sample, called the convalescent phase blood sample was collected from these patients 10-14 days following the first extraction except from one of the students from whom throat washings was obtained.

The four medical students as well as the B.S. Hygiene student were seen only after they had fully recovered from their illnesses; hence, only the convalscent phase blood sample was extracted from them. Blood collected from these five students at the beginning of the school year was used in place of the acute phase sample. There were therefore a total of 93 paired blood samples.

All throat washings obtained were iced in transit to the laboratory where they were kept in a Revco freezer at approximately minus 60°C. The blood samples were allowed to clot and the serum was separated and stored in an ordinary freezer at minus 10°C until the time when they were tested.

Serological Test

To determine whether a strain of influenza virus was the etiologic agent involved in this epidemic, a test run employing the hemagglutination-inhibition (HI) test (1) on 3 paired serum samples was performed. The antigens used in the test were A/FMI/47; A/PR8/34; A/Formosa 303/57; a strain closely related to Swine strain isolated here in 1957; and B/Lee/40. None of the 3 paired serum samples tested showed a significant antibody increase against any of the antigens used. It was felt that failure to obtain a significant antibody increase in all of the three paired serum samples might have been due to the improper selection of antigens used.

Another run on 3 other paired serum samples was done, this time using all of 15 strains of influenza virus available in our laboratory. In addition to those already mentioned, the following strains were included.

A/WS/33	A/Denver/57
A/Conley/54	B/Great Lakes/54
A/Valley Forge/57	C/1233/49
A/Rhode Island/57	D/Sendai/52
A/AA	A strain similar to Japan 305 isolated here in 1957.

Except for the two strains isolated locally, all other strains were obtained from the United States.

Two of the 3 paired samples showed a significant increase in antibody titer against strains of influenza belonging to Type A. One of the two samples showed significant increase in antihemagglutinins for WS, FM1, PR8 and Japan 305 strains. The other paired sample gave a significant antibody increase for FM1 and Japan 305 strains. The third sample did not show any significant antibody increase against any of the antigens used.

With these results, we felt justified to continue the HI test on all remaining serum samples including the 3 samples used in the first run. To save on materials and to simplify the work, only 5 strains of influenza virus belonging to Type A were used; namely, WS, PR8, FM1, Japan 305 and Denver. These 5 strains were chosen on the basis of the results of the preliminary HI test run which showed significant antibody response to these strains.

Virus Isolation

Attempts to isolate virus from all seven specimens of the throat washings were made employing a method similar to that used in a previous study (2). All amniotic fluids obtained were tested for the presence of an hemagglutinating agent with guinea pig red blood cells as well as chicken red blood cells. Parallel tests at room and refrigerator temperature were done. Results were considered negative upon failure to recover an hemagglutinating agent after three successive amniotic passages. Amniotic fluid pools from which an hemagglutinating

agent was recovered were subsequently passed into the allantoic cavity of embryonated eggs. Further allantoic passages were undertaken until the virus had reached a titer sufficiently high for identification of the specific strain.

Typing serums from roosters or large hens were prepared according to the method described by Jensen (1). Identification of the agents obtained from embryonated eggs was done by the hemagglutination-inhibition test (HI). Immune serums prepared against PR8, FM1, Japan 305, Denver and Swine strains were used. Two-fold serial dilutions of each serum in saline, starting with a 1:50 dilution and ending with a 1:25,600 dilution, were employed in the test.

RESULTS

Out of a total of 93 paired serum samples tested, 31 or one-third showed a significant increase in antibody titer against one or more of the five strains of influenza virus Type A.

As shown in Table 1 there were 14 serum samples registering significant antibody increases against each of the newer Type A influenza viruses — A/Japan 305 and A/Denver — than against the older strains of Type A.

There were only 4 paired samples showing significant antihemagglutinin increase against A/WS; 9 against A/PR8; and 10 against A/FM1. It is also to be noted that the antibody increase against the newer Type A strains were generally higher than against the older strains of the same type.

Seventeen of the 31 individuals with significant HI results registered significant antibody increase against more than one of the antigens used while 14 showed a monotypic response. The frequency of significant responses to the different antigens used in the test can be seen in Table 2.

Four out of the 7 throat washings inoculated in embryonated eggs yielded an hemagglutinating agent.

As shown in Table 3 one of the individuals from whom an hemagglutinating agent was recovered had a significant increase in antihemagglutinins against A/FM1; another against

both A/Japan 305 and A/Denver strains; the third against A/FM1, A/PR8 and A/Japan 305. Antibody response of the fourth individual was not determined because there was no paired blood sample.

Table 1. DEGREE OF ANTIBODY RISE IN 31 PATIENTS WITH SIGNIFICANT HI TEST RESULTS
(July 8 — September 15, 1958)

Patient Code No.	Age in Years	WS	PR8	FM1	305 Japan	Denver
1334*	20	—	—	4-fold	8-fold	—
1361**	14	4-fold	8-fold	—	32-fold	—
1367**	23	—	—	4-fold	—	—
1370	20	—	—	4-fold	4-fold	—
1371	12	—	—	4-fold	—	4-fold
1377	20	—	—	—	4-fold	16-fold
1380	21	4-fold	—	16-fold	4-fold	—
1381	17	—	—	—	—	—
1388***	19	—	4-fold	4-fold	16-fold	4-fold
1393	22	4-fold	—	—	—	—
1395***	17	—	—	—	—	4-fold
1396	20	—	4-fold	—	—	4-fold
1398	18	—	—	4-fold	—	16-fold
1402	16	—	4-fold	—	—	—
1403	22	—	4-fold	—	4-fold	—
1405	21	—	—	—	4-fold	16-fold
1407	17		8-fold	—	4-fold	—
1409	30	—	—	—	—	8-fold
1410***	17	—	—	—	—	4-fold
1413	17	—	8-fold	—	—	—
1444	21	—	—	—	—	4-fold
1423	20	—	—	—	8-fold	8-fold
1445	24	—	—	—	—	64-fold
1453	15	4-fold	—	—	4-fold	—
1456	23	—	4-fold	4-fold	—	—
1460	19	—	—	—	8-fold	4-fold
1470***	22	—	—	—	—	—
1473	16	—	—	—	—	8-fold
1475	25	—	—	8-fold	4-fold	—
1489	21	—	—	8-fold	—	—
		—	4-fold	16-fold	—	—
Total		4	9	10	14	14

* Included in first test run
** Included in second test run
*** With history of influenza in 1957

Table 2. DISTRIBUTION OF HEMAGGLUTINATION-INHIBITION TEST TO RESULTS IN 93 INFLUENZA SUSPECTS ACCORDING TO THE ANTIGENS AGAINST WHICH SIGNIFICANT RISE IN ANTIBODY TITER WAS DEMONSTRATED
(July 8 — August 10, 1958)

Antigens					
WS	FM1	PR8	Japan 305	Denver	Number of subjects
+	+	+	+	−	1
−	+	+	−	+	1
−	+	+	−	−	1
−	+	−	+	−	3
−	+	−	−	+	1
−	−	+	+	−	2
−	−	+	−	+	1
−	−	−	+	+	4
+	−	−	+	−	2
+	+	−	−	−	1
−	+	−	−	−	2
−	−	+	−	−	3
−	−	−	+	−	2
−	−	−	−	+	7
−	−	−	−	−	62
Total 4	10	9	14	14	93

Table 3. ANTIHEMAGGLUTININ RESPONSE OF INDIVIDUALS FROM WHOM ISOLATION OF VIRAL AGENT WAS ATTEMPTED
(July 8 — August 10, 1958)

Patient Code No.	Result of isolation	HI response to antigens				
		FM1	PR8	Japan 305	Denver	WS
1370	Positive	+	−	−	−	−
1371	Positive	−	−	+	+	−
1361	Positive	+	+	+	−	−
1357	Positive	No paired blood sample				
1359	Negative	−	−	−	−	−
1360	Negative	−	−	−	−	−
1350	Negative	−	−	−	−	−

+ Significant antibody increase
− No significant antibody increase

Identification of the isolates by HI test gave the results shown in Table 4.

Table 4. IDENTIFICATION OF THE VIRUS ISOLATES BY HI TEST USING CHICKEN ANTISERUM*

Virus Isolate Code Number	PR8/34	FM1/47	Japan 305/57**	Denver/57
1357	50***	50***	400	50
1361	50***	50***	800	200
1370	50***	50***	400	50
1371	50***	50***	1600	100

* Titers expressed as reciprocals of initial serum dilutions.

** Strain isolated locally in the epidemic of 1957.

*** Less than 50.

DISCUSSION

The results obtained by the various tests employed in this study leave no doubt that there was an epidemic of influenza. The results of the HI test alone was sufficient evidence of the existence of the epidemic. The information obtained by this test showed that Type A influenza virus was responsible for the epidemic since significant antibody rise was noted only against this type and not against types B, C or D. It was not easy, however, to define with certainty the precise immunologic strain involved in this outbreak from the results of the HI test alone since more than half of the individuals included in this study showed a heterotypic antibody response to antigenically related strains.

The relatively small proportion of individuals showing a positive serological response is not surprising since a similar observation was noted among cases studied abroad during the epidemic of influenza in 1957 (3). The use of the complement-fixation test would have probably enabled us to detect more paired blood samples with significant increase in antihemagglutinins but the lack of antigens at the time this study was conducted precluded the use of such a test.

The heterotypic responses noted in a number of individuals can be ascribed to a recapitulation of experiences in the past with antigenically related strains resulting in the reinforcement of the primary antibody as well as the broadening of the anti-

body spectrum. Such an occurrence can be summarized by the expression "the doctrine of original antigenic sin" so called by Davenport (4).

While the results of the HI test does not permit one to draw definite conclusions regarding the precise immunologic strain of influenza virus involved in this epidemic, the result of the study on the isolation of etiologic agent gives more information in this respect. Identification of the 4 hemagglutinating agents obtained show that they belong to the Far East strains of influenza virus and antigenically similar to A/Japan 305/57 and to a slight extent to A/Denver/57.

As suggested by Dr. C. H. Andrewes (5) of the World Influenza Center, we propose to call these 4 strains A/Philippines 1-4/58.

SUMMARY

Ninety-three paired blood samples collected from cases with clinical illnesses resembling influenza were tested for evidence of antihemagglutinin response against WS, PR8, FM1, Japan 305 and Denver strains of Type A influenza virus.

Thirty-one paired samples showed significant antibody increase to one or more of the antigens used. In general, there were more significant and higher antibody responses against the newer Type A strain — Japan 305 and Denver — than to the older WS, FM1 and PR8 strains. Significant antibody increase to the older Type A strains can be ascribed to effects on the antibody-forming mechanisms of antigenically related strains during childhood.

Four hemagglutinating agents were isolated from 7 throat washings. HI test with chicken antiserums reveal that these are closely related to the Far East strains A/Japan 305/57 and to a lesser extent to A/Denver/57.

These new isolates have been designated A/Philippines 1-4/58.

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