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Antigenic Variation of Asian Influenza Virus.* (24354)

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During the fall of 1957, there was an outbreak of Asian influenza at the State University of Iowa. Numerous paired human sera were collected and 20 virus isolations made. All isolates were identified as Asian or "A" virus. Many of patients' paired sera gave diagnostic rises in titer by complement fixation test (CF), but were negative by the hemagglutination inhibition test (HI) when A/Jap/305/57 virus was used as antigen. To ascertain whether a more satisfactory HI antigen was available, several early isolates were tested. There was little cross reaction by HI among these viruses against the same patients' sera and many sera previously negative by HI were positive. These results suggested that "A" viruses isolated were antigenically different, and that cross neutralization by HI should show this difference in antigenicity. This paper summarizes results of cross neutralization by HI of 19 of the "A" virus isolates, and the A/Jap/305/57 virus with 20 paired human sera.

Materials and methods. Viruses. "A" viruses were recovered from students at the State University of Iowa by use of embryonated eggs. Twenty strains were recovered and designated by abbreviation of patients' names. One strain A/Jap/305/57 was supplied by the Communicable Disease Center. *Hemagglutinating antigens.* The antigens were allantoic fluids from embryonated eggs infected with "A" viruses. The allantoic fluid antigens after 4 to 6 passages had titers ranging from 1:80 to 1:2560 against chicken red blood cells. Allantoic fluid virus from the same egg passage was used as antigen throughout the tests. Each antigen was bacteriologically sterile. *Paired sera from influenza patients.* All paired human sera, designated by abbreviation patients' names from whom they were drawn, were inactivated at 56°C for 30 min. and used without further treatment. *Serological procedures.* Hemagglutination inhibition (HI) titrations were done with chicken red blood cells using the Salk pattern test(1), with one modification. Instead of 0.5% chicken red blood cell suspension, a suspension of 0.25% cells was used. Titers for the HI test are reported in terms of initial serum dilution (1:10) and all titers adjusted for 4 hemagglutinating doses. A standard complement fixation test (CF) was used(1). All paired sera tested, except PET, showed at least a 4-fold rise in titer. PET sera showed a 2-fold rise.

Results. Twenty "A" viruses were tested against 20 paired human sera by HI. From results obtained, the viruses were classified as 2 main groups: (1) those against which both acute and convalescent sera had high HI titers and which were considered inhibitor susceptible, (2) those against which the acute

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TABLE I. Cross Neutralization of Human Paired Sera with "A" Viruses by Hemagglutination Inhibition.

		Inhibitor insusceptible viruses											
		A JAP	JOS	JOY	SIM	THU	DAU	SWE	TJA	DEN	JOH	PEP	THO
Sera		Hemagglutination inhibition titers											
AIS	A*	0†	0	0	‡		0	0	0	0	<15	0	<15
	C	"	10	"			20	"	10	40	60	40	15
BED	A	"	0	"			0	"	0	0	0	0	0
	C	160	320	160			640	80	320	2560	640	80	640
COO	A	0	0	0	0	0	0	0	0	0	0	0	0
	C	160	320	320	160	320	2560	40	320	5120	320	160	640
FUR	A	0	0	0	0	0	0	0	0	0	0	0	0
	C	"	40	80	20	20	80	80	20	640	80	80	80
GRE	A	"	0	0			0	0	0	0	0	0	0
	C	"	20	20			20	10	20	320	320	160	40
KIM	A	"	0	0	0	0	0	0	0	0	0	0	0
	C	"	"	"	10	"	320	"	10	160	20	20	40
LAH	A	"	"	"	0	"	0	"	0	0	0	0	0
	C	160	160	1280	320	320	1280	640	320	2560	640	640	160
LAT	A	0	0	0	0	0	0	0	0	0	0	0	0
	C	40	"	"	10	"	"	"	"	20	20	80	40
MAR	A	0	"	"			"	"	"	0	0	0	0
	C	"	20	160			160	40	40	640	160	40	160
NOR	A	"	0	0	0	"	0	0	0	0	0	0	0
	C	"	"	"	"	"	"	"	"	10	20	20	10
PEP	A	"	"	"	"	"	"	"	"	0	0	0	0
	C	10	"	"	"	"	"	10	"	20	20	"	10
PET	A	0	"	"	"	"	"	0	"	0	0	"	0
	C	"	"	"	"	"	"	"	"	"	"	"	"
PETS	A	"	"	"	"	"	"	"	"	"	"	"	"
	C	"	"	10	10	"	20	40	10	80	80	20	40
REE	A	"	"	0		"	0	0	0	0	0	0	0
	C	10	20	"		"	40	20	10	320	40	80	40
SCH	A	0	0	"	0	"	0	0	0	0	0	0	0
	C	"	"	"	"	"	20	"	10	80	40	80	20
SWE	A	"	"	"	"	"	0	"	0	0	0	0	0
	C	"	"	"	10	"	20	20	"	10	20	"	20
VEE	A	"	"	"		"	0	0	"	0	0	"	0
	C	"	10	"		"	20	40	10	320	80	40	80
WEN	A	"	0	"			0	0	0	0	0	0	0
	C	"	"	"			20	20	10	320	160	80	80
WHI	A	"	"	"	0	"	0	0	0	0	0	0	0
	C	"	"	"	"	"	"	40	"	40	20	20	10
WIL	A	"	"	"	"	"	"	0	"	0	0	0	0
	C	"	"	"	"	"	"	160	"	"	10	10	"

* A = Acute; C = Convalescent. † 0 = <10. ‡ No more sera available.

sera had no titer and were considered inhibitor insusceptible. Because both acute and convalescent sera had high titers against inhibitor susceptible viruses, a diagnostic rise was difficult to obtain. Consequently, in Table I only results using inhibitor insusceptible viruses are shown. All inhibitor insusceptible strains tested showed that patients had no "A" HI antibody previous to infection with "A" virus.

Table II illustrates progressive increase in number of sera with detectable HI antibody using different "A" viruses as antigens.

Nineteen of the sera tested had both a 4-fold rise in CF titer and detectable HI antibodies. PET sera was HI negative against all "A" viruses, including the PET virus. In another instance not included in the Tables, an "A" virus was isolated from a patient whose paired sera was HI negative even against his

TABLE II. Comparison of Number of Hemagglutination Inhibition Antibody Responses to 12 "A" Viruses.

Virus	No. sera* showing detectable HI antibodies	% positive diagnosis by HI
THU	3/15	20
A Jap	6/20	30
JOY	7/20	35
JOS	9/20	45
SIM	7/13	53
TJA	13/20	65
DAU	14/20	70
SWE	14/20	70
PEP	17/20	85
DEN	18/20	90
THO	18/20	90
JOH	19/20	95

* 20 sera maximum number tested.

own virus. However, a third blood specimen drawn 1 week later, on the twenty-first day after onset, did show a diagnostic HI titer against several "A" viruses. Perhaps the PET convalescent sera was also taken too early.

Discussion. Jansen and Hogan(2) found that out of 123 cases diagnosed as Asian influenza only 47 had diagnostic rises in titer by both CF and HI. They also found that 29/123 cases were CF negative and HI positive, and that 47/123 cases were CF positive and HI negative. The poor agreement between the CF and HI tests may be explained by our results. The results in Table I show that many sera negative by HI against one "A" virus, were positive by HI when other "A" viruses were used as antigen. If PET sera were excluded, 19/19 of the sera tested showed HI antibody titers against JOH virus. Use of JOH virus as the HI antigen made the HI and CF tests equally reliable.

The discrepancy among "A" viruses in ability to detect HI antibodies (Table II) indicated the necessity for testing more than one "A" virus as an antigen. One wonders how many sera reported negative by HI during the recent Asian inflfluenza epidemics would have been positive with use of several "A" viruses as antigens.

The logical explanation for the variance in the "A" viruses was that some of these viruses were antigenically different. Widelock *et al.* (3) isolated several "A" viruses late in an epidemic in N. Y. City which differed from the majority of strains in that they cross reacted with FM_1 sera as well as showing titers against anti-Asian sera. Takatsy, and Barb (4) found by use of hyperimmune chicken sera differences between the A-Singapore/1/57 virus and "A" viruses isolated in Hungary.

Our results showed that within a small area over a period of 2 to 3 months there appeared to be a wide antigenic variation among "A" viruses isolated. This variation was demonstrated by use of patients' sera drawn during the same epidemic. If all "A" viruses were antigenically identical, then all strains could be used to detect equally well the HI antibody present in a patient's convalescent serum.

The differences in HI titers could not be due to some patients being poor antibody producers because some sera, for example, WHI and WIL failed to show HI antibody rises against most viruses, but against at least one of the viruses there was a good rise. If they were poor antibody producers against some, why not against all? The immunological background of the patient, *i.e.* strains of influenza virus that infected him in the past, may be advanced to explain the variable antibody response. Unless one admits to antigenic differences among individual strains of "A" virus, it is difficult to explain why the same patient responds differently serologically to different strains used in the test. The immunological background of a single patient is the same. The difference must be in the test viruses.

Another possible explanation for antigenic diversity of "A" viruses could be the P-Q-R variation of van der Veen and Mulder(5). They have described P, Q, and R strains of influenza viruses of the same antigenic type. P strains had high HI titers only with homologous sera. Q strains had low HI titers and R strain high HI titers with both homologous and heterologous sera.

Levy and Wagner(6) reported that Baltimore strains of Asian influenza virus isolated in Oct. 1957 were in the R phase while the A/Jap/305/57 virus isolated 7 months previously was in the Q phase. This explained why the Baltimore viruses were better HI antigens than the A/Jap/305/57 virus.

However, our results were not compatible with P-Q-R variation. Several viruses, for example JOY virus, produced low HI titers with most sera, but high HI titers with the remainder. PEP virus showed no HI titer with PEP sera, but detected HI antibodies in 17 of the 19 other sera. In what phase were these viruses? Furthermore, at the beginning of the outbreak at SUI several viruses, THO, THU, SIM, and SWE, were all isolated within a week of each other. Yet there was wide variation in ability of these viruses to detect HI antibody (Table II). It would be difficult to say that these viruses were in any one phase.

A quantitative rather than qualitative difference in viral antigens might be postulated. If this were true, then the highest HI titers should occur when paired sera were tested against the homologous virus. SWE virus against SWE sera gave HI titers which did not exceed those of several other viruses. PEP virus against PEP sera showed no HI titer, while 3 other viruses did detect HI antibody in the convalescent serum.

The failure of homologous virus to produce highest HI titer, could be the result of a change in antigenicity *in vivo*. PEP virus, for example, might have had a poor surface HI antigen and a good HI antigen within the virus. After infection, the breakdown of viral particles would allow the HI antigen previously masked by surface antigen to stimulate production of HI antibodies. Sera from the patient could not react *in vitro* with PEP virus because of the surface antigen, but other "A" viruses with the right surface antigen would react.

Adsorption experiments are needed to definitely determine whether "A" viruses are qualitatively distinct. Cross adsorption studies are under investigation.

It was not determined why some of these strains were inhibitor susceptible and others insusceptible. However, use of inhibitor insusceptible viruses as HI antigens removed the troublesome problem of inhibitors without resorting to chemical treatment of either sera or virus.

Since this paper was writen, Purnell, Osterhout, Tamm(7) reported that 5 strains of "A" virus isolated at the Hospital of Rockefeller Institute were closely related among themselves by cross hemagglutination inhibition. Our results did not show this antigenic homogeneity.

Summary. Asian or "A" viruses isolated at the State University of Iowa in the recent epidemic were tested against selected paired human sera. All sera which had a 4-fold rise in titer by the complement fixation test also had a significant rise in titer by the hemagglutination inhibition test when several "A" viruses were used as antigens. Neither viruses nor sera were chemically treated. There was a distinct variation in antibody response to "A" viruses when tested by hemagglutination inhibition. Possible causes of this variation have been discussed.

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