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Recombination and Transmission Studies with Influenza Virus (U)

ANNUAL PROGRESS REPORT

by

Edwin D. Kilbourne, M. D.

September 1970
(For the period 1 June 1969 to 1 August 1970)

Supported by
U. S. Army Medical Research and Development Command
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Contract No. DA-49-193-MD-2795

Mount Sinai School of Medicine
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SUMMARY

A recombinant influenza virus X-31 which is antigenically identical with the new Hong Kong (HK) variants but which grows to high yields in chick embryos, has been produced by hybridization of the standard Aichi vaccine strain with A_0/PR8 virus. With this virus, purified inactivated vaccine was produced by Evans Medical Ltd. in England for clinical trials in the United States under the auspices of the Commission on Influenza.

Correlative and comprehensive studies of the X-31 vaccine and standard HK/Aichi vaccine of matched CCA activity have been conducted in mice, rabbits and man in six different laboratories. In these studies advantage has been taken of the availability of unique recombinant viruses for serologic analyses which permit discrimination between hemagglutinin and neuraminidase antibody response to vaccination. The following conclusions can be drawn from these combined studies of identical vaccine lots:

I. Both Aichi and X-31 vaccines stimulated production of neutralizing antihemagglutinin and antineuraminidase antibodies in mice, rabbits and man. A trend in favor of X-31 was consistently observed in all 3 host systems.

II. Significant protection of volunteers from experimental influenza was afforded by both vaccines as determined by reduction in illness and shedding of virus after challenge.

III. Studies of military recruits at Fort Ord demonstrated the efficacy of X-31 vaccine in preventing natural infection under field conditions. While 104 hospitalized cases of influenza were found among the 7,934 controls (1.3%), only 6 (0.36%) occurred in the 1,682 vaccinees ($p \approx <0.002$).

IV. The feasibility of utilizing a recombinant "pre-fabricated" virus for commercial scale vaccine production has been demonstrated for the first time.

V. Immunogenicity studies in experimental animals correlated well with antigenicity and protection data in man. Indeed, it was possible to distinguish between the two vaccines on the basis of their relative effects on human and mouse ID_{50} and their capacity to stimulate the production of hemagglutination-inhibiting, neutralizing and plaque-inhibiting antibody.

The differing immunogenicity of the two vaccines was not predictable from their CCA concentrations, which did not differ significantly.

CONTENTS

INTRODUCTION

Although other studies of influenza virus antigenicity have been conducted in this laboratory during the past year, the present report will be devoted entirely to the results of correlative and comprehensive studies of a recombinant influenza virus vaccine (X-31) in man and experimental animals. This study had four principal goals, all of which have been achieved:

I. To demonstrate the feasibility of rapidly producing and employing in commercial vaccine production a recombinant influenza virus of requisite antigenicity and growth characteristics (i.e., yield in the chick embryo);

II. To test such a vaccine in man to determine its antigenicity and efficacy in comparison with a "standard" monovalent vaccine;

III. To correlate the results of field trials with concomitant tests of immunogenicity in mice and rabbits; and

IV. To measure response to immunization with respect to antibodies to both influenza virus envelope proteins, hemagglutinin and neuraminidase. [Antibodies to the viral neuraminidase have recently been shown in this laboratory to be important in immunity (Schulman, et al., J. Virol., 1968, 2:778) and to appear in man following natural infection (Kilbourne, et al., J. Virol., 1968, 2:761)]

Preliminary studies of antigenicity in man and the immunogenicity studies in mice and rabbits were conducted in this laboratory. Tests of the X-31 and commercial Aichi vaccines in experimental infection in volunteers were conducted by Drs. Couch and Douglas of Baylor College of Medicine. Field trials of the X-31 vaccine were conducted in military recruits at Fort Ord, California, through the combined efforts of the Preventive Medicine Division, U. S. Army Hospital, Fort Ord, and the Sixth U. S. Army laboratory. The request to conduct the study originated from the Commission on Influenza of the Armed Forces Epidemiological Board and was actively supported by the Surgeon General with the approval of the Army Investigational Drug Review Board.

Support and assistance for these studies was also provided by the Vaccine Development Branch of the National Institute of Allergy and Infectious Disease.

The principal investigator is also indebted to Dr. Nicholas Tauraso of the Division of Biologic Standards (DBS) for provision of the standard Aichi vaccine used in these studies, and to Dr. Tauraso and

Dr. F. M. Davenport for determination of the CCA unitage of both the X-31 and the Aichi vaccines.

The study would not have been possible except for the enterprise and generosity of Dr. David Breeze of Evans Medical Limited who produced and provided the 3,000 doses of X-31 vaccine employed in these investigations.

Recombination of A_0 PR8 34 and HK Aichi 68 virus to produce X-31

The principle had been established in studies published from this laboratory in 1960 (Kilbourne and Murphy, J. Exper. Med., 1960, 111:387) that mixed infection of the chick embryo allantoic sac with low yielding wild type (A_e) virus and a standard laboratory A_0 PR8 virus could result in a stable hybrid or recombinant virus of wild type antigenicity and PR8 growth capacity.

When a strain of Hong Kong virus (HK/16/68) became available to us on September 20, 1968, we essayed a similar experiment immediately and isolated (in less than 2 weeks) a Hong Kong-like recombinant of increased growth capacity (X-30), the virus was said to be inacceptable for vaccine production (although it exceeded by four to six fold the titer of standard Aichi strains then in use in commercial vaccine production) because the HK parental virus had a history of monkey kidney passage.

Accordingly, the experiment was repeated - this time with HK/Aichi strain in early passage obtained directly from the DBS and the A_0 PR8 34 strain (used in earlier vaccine production) from Dr. F. M. Davenport, Director of the Commission on Influenza of the AFEB. The results of this experiment, initiated on February 4, 1969, are summarized in Table 1. Prior partial thermal inactivation of HK/Aichi permitted the injection of less than one EID_{50} (but $10^{5.2}$ inactivated particles with potential genetic activity) thus facilitating the subsequent selection of a recombinant of identical serotype. Parallel passages of inactived HK/Aichi alone (passages 1b and 2b) verified the absence of infective virus in the inoculum. Following passage with A_0 PR8 antiserum, hemagglutinating virus was detected only in chick embryos that had been mixedly infected (passage 2a) but not in control passages of inactivated HK/Aichi (2b) or A_0 PR8 (2c) alone. Again, a high titer recombinant (X-31) was isolated and identified serologically within two weeks. After final cloning (passages 3 - 5a) the virus was available for pilot vaccine production on March 17, 1969. On April 9, 1969, a sample was sent to Dr. David Breeze of Evans Medical Ltd., who had been experimenting with production of the X-30 recombinant previously isolated. On May 5, 1969, Dr. Breeze reported to us that recombinant X-31 was growing exceptionally well and exceeded 3840 - 5120 HA units per 0.25 ml. compared to titers of 1280 - 2560 per 0.25 ml. for his own Hong Kong production strain. Pilot production was then started. Batch concentrates of the first lot were tested at Dr. Davenport's laboratory for CCA titer on July 18, 1969,

Table 1

Recombination of A_0/PR8 and HK/Aichi to produce a high yielding virus of HK serotype, X-31

passage	mixed or single infection of chick embryos
1a	Δ HK(Aichi)* (<1 EID_{50}) x A_0/PR8xx (5 EID_{50})
1b	Δ HK(Aichi)* (<1 EID_{50})
1c	A_0/PR8xx (5 EID_{50})
	passage with A_0 PR8 antiserum
2a	HK - like virus "X-31"
2b	no detectable virus
2c	no detectable virus
3-5a	virus cloned at 2 successive limiting dilutions of 10^{-9} and 10^{-10} after passage at 10^{-6}

* HK/Aichi/2/68 obtained from division of Biologic Standard courtesy of Dr. Nicholas Tauraso (6th chick embryo passage). Inactivated at 37°C for 96 hours with reduction in infectivity titer from 10^{-9} EID_{50}/0.1 ml to $10^{-3.8}$ EID_{50}/0.1 ml.

xx A_0/PR8/34 obtained courtesy of Dr. F. M. Davenport; passage history: ferret 198, mouse 593, chick embryo 175.

Table 2

PROPERTIES OF X-31 AND PARENTAL VIRUSES

Virus	Antigens[a]		Yield in eggs (HA titer)	Optimal growth at 35°C	Viral morphology	Elution from erythrocytes[b]
	Hemagglutinin	Neuraminidase				
PR/8	PR/8	PR/8	8192	+	Spherical	+
HK[c]	HK	HK	256	-	Filamentous	-
X-31	HK	HK	8192	+	Spherical	+

a Antigenic characterization defined by hemagglutination inhibition, neuraminidase inhibition and plaque inhibition

b Difference first noted by Dr. Geoffrey Schild

c HK = Division of Biologics Standards 5th-egg passage Aichi strain - no monkey kidney passage

From Kilbourne, E. D., Bull. WHO 1969, 41:643

and the results communicated to Dr. Breeze so that he might make appropriate dilutions before packaging of the vaccine. 3,000 1 ml. doses of vaccine in single dose ampules were received by Dr. Daniel Mullally of the Vaccine Development Branch for distribution on November 1, 1969. The CCA titers of the packaged vaccine and a standard Lilly control Aichi vaccine were again checked by Dr. Davenport's lab and also by Dr. Tauraso of the DBS. Results are presented below.

Characterization of X-31 and parental viruses

A summary of the comparative properties of X-31 and parental viruses is presented in Table 2. It will be noted that X-31 is indistinguishable antigenically from the Aichi strain but is similar in morphology, growth and red cell elution characteristics to the PR8 parent.

Vaccine preparations

1) X-31 (A_2/HK/X31/68 - Batch No. 1) This vaccine was prepared by Evans Medical Ltd. by a process involving centrifugation through sucrose in an initial bach of 3625 ml. Virus was suspended in M/100 phosphate buffered saline. Inactivation was by formalin and betapropriolactone. The calculated CCA unitage was 600 CCA/ml.

2) Aichi - (A_2/HK/Aichi/68 - Lot. No. 2MT62) This vaccine was prepared by Eli Lilly and Co. by zonal centrifugation, and formalin inactivated. Labelled CCA unitage was 800 CCA per ml.

3) B/Massachusetts/3/66 - Eli Lilly and Co. Lot No. 2MT11. A zonal centrifuged vaccine, formalin inactivated. Labelled CCA unitage 800 CCA per ml. (This vaccine was used only in mouse experiments as a control inoculum.)

The actual CCA unitage of the influenza A vaccine was determined independently by the Ann Arbor and DBS laboratories in replicate determinations. The values are indicated below.

(data of Hennessey and Davenport)

date of test	Vaccine Aichi Lilly	X-31
10/31/69	561 CCA	526
11/ 3/69	475	558
11/ 4/69	415	504
11/ 6/69	558	542
mean	502	532

(data of Tauraso (DBS)

10/31/69	555	719
11/ 4/69	567	615
11/ 5/69	400	499
11/ 5/69	541	521
11/ 6/69	449	525
mean	504	576
overall mean	503	556
SD	67	70

.2>P>.1

Summary

Vaccine	Hemagglutinating Activity of Vaccine: CCA units/ml. (label)	CCA units/ml. (actual)	HA TITER* chick RBC	HA TITER* human RBC	Neuraminidase Activity nM/min./ml of NANA released**
Aichi	800	503	3072	3072	35.6
X-31	600	556	8192	8192	22.6

* by fractional dilution technique of Tamm and Horsfall
** mean of 4 determinations

Other viruses used in the study

The serologic analysis of antigenic response was facilitated by the use of certain recombinant viruses in which the hemagglutinin (H) and neuraminidase (N) antigens of the HK virus had been dissociated. These viruses have been identified in previous reports and publications in simplistic shorthand or laboratory vernacular. Because we now recognize the necessity of distinguishing antigenic variation within

Table 3

Explanatory Code for Wild Type and Recombinant Viruses used in Serologic Analysis in the present Study

H = Hemagglutinin Antigen

N = Neuraminidase Antigen

Vernacular	Antigenic designation based on H and N antigens
Aichi	H_{hk} N_{hk} [or HKE(HK)]
X-31	H_{hk} N_{hk} [" "]
HKe	H_{hk} $N_{A_0/nws}$
A_0E(HK)	$H_{A_0/nws}$ N_{hk}
X-7	$H_{A_0/nws}$ $N_{A_2/ri/5'+}$ [or $A_0E(A_2^+)$]
X-15	$H_{equi\ 1}$ $N_{A_2/ri/5'+}$
X-15(HK)	$H_{equi\ 1}$ N_{hk}

N.B. HK and A_2 hemagglutinins are considered to be equivalent to separate subtypes (Schulman & Kilbourne, Proc. Nat. Acad. Sci. (1969) 63: 326).

There is some antigenic cross reactivity of early (A_2/RI/5$^+$/57) and HK virus neuraminidases and complete identity of HK and late A_2 virus neuraminidases (Schulman & Kilbourne, Bull. WHO (1969) 41:425).

subtype designations of both H and N proteins, we now employ a more formal and precise (if more cumbersome) terminology for both wild type and recombinant viruses. This formal taxonomy for viruses used in the present study is presented in Table 3. The new designation for X-7, HA_0 nws, NA_2 ri_5+, indicates that this recombinant contains the hemagglutinin of A_0NWS and A_2 neuraminidase derived from the A_2/RI/5+ strain.

Immunization of mice with X-31 and Aichi vaccines

Groups of mice were immunized intraperitoneally with 5 fold dilutions of X-31 and Aichi virus vaccines. Control animals were injected with influenza B vaccine. Ten animals in each group were challenged by aerosols of serially diluted unadapted Hong Kong virus and were autopsied 2 days later to determine the proportion of mice in each group infected with each virus concentration (MID_{50}). The remaining animals were challenged with mouse-adapted Aichi virus. Pulmonary virus titers were measured in 5 animals in each group 3 days after challenge and lung lesions were assessed 7 days after challenge in 5 others.

The results of these experiments are shown in Tables 4 - 6 and may be summarized as follows:

1) HI antibody titers against Aichi virus were higher in animals immunized with X-31 vaccine than in mice immunized with equivalent doses of Aichi vaccine (Table 4). Furthermore, no end point was reached with X-31 immunization, whereas animals immunized with the 2 lowest doses of Aichi vaccine failed to develop HI antibody to Aichi virus. Similarly, when the same sera were examined in plaque inhibition tests (Table 5), pooled sera from animals immunized with the 3 lowest doses of Aichi vaccine had no demonstrable plaque inhibiting antibody, whereas plaque inhibiting antibody was demonstrable in the sera of mice immunized with even the most dilute concentration of X-31 vaccine.

2) The 2 vaccines were of equal potency with respect to their capacity to stimulate antineuraminidase antibody in mice.

a) HI antibody titers against X-15(HK) (Equi, hemagglutinin - HK neuraminidase) were equivalent in animals given equivalent doses of the 2 vaccines (Table 4).

b) Following immunization with equivalent doses of X-31 or Aichi vaccines, no differences were observed in enzyme inhibiting or plaque size reducing antibody titers [tested against A_0E(HK)] - Table 5.

3) The effects of vaccine dose on susceptibility to the initiation of infection are shown in Table 6 (MID_{50}). With both vaccines, larger immunizing doses increased the quantity of aerosolized virus (decreased

TABLE 4

Serum Hemagglutinating-Inhibiting antibody response in Mice Immunized with Five Fold Dilutions of X-31 and Aichi Vaccines.

Vaccine		Test Antigen: Aichi	HKe	X-15 (HK)	X-15
X-31	1:2	3.8*	2.2	4.2	<1.0
	1:10	2.0	2.0	2.8	<1.0
	1:50	2.2	1.0	1.4	<1.0
	1:250	2.0	0.8	1.4	<1.0
Aichi	1:2	2.2	1.6	3.6	<1.0
	1:10	1.2	1.2	1.6	<1.0
	1:50	<1.0	<1.0	0.8	<1.0
	1:250	<1.0	<1.0	1.0	<1.0
B		<1.0	<1.0	1.0	<1.0

* reciprocal of serum dilution which inhibits hemagglutination. Initial serum dilution = 1:10. Mean titer ($\log_2$) of individual titrations of 5 sera in each group.

TABLE 5

Enzyme Inhibiting (EI) Plaque Inhibiting (PI) and Plaque Size Reducing (PSR) Antibody Titers in Sera of Mice* Immunized with Five Fold Dilutions of X-31 and Aichi Vaccines

		EI	PI		PSR
Vaccine Group		A_0E (HK)	Aichi	HKe	A_0E (HK)
X-31	1:2	1:16	3200	800	800
	1:10	<1:8	600	<400	<400
	1:50	<1:8	400	<400	<400
	1:250	<1:8	400	<400	<400
Aichi	1:2	1:12	1600	400	800
	1:10	<1:8	<400	<400	<400
	1:50	<1:8	<400	<400	<400
	1:250	<1:8	<400	<400	<40[illegible]
B		<1:8	<400	<400	<400

* pooled sera; 5 mice in each group.

TABLE 6

Effects of Immunization of Mice with Five Fold Dilutions of X-31 and Aichi Vaccines or Susceptibility to Initiation of Infection and Pulmonary Virus titers and Lung Lesions Following Infection with Hong Kong Virus.

Vaccine Group		MID_{50}*	Pulmonary Virus Titers (day 3)**	Lung Lesions (%) (day 7)†
X-31	1:2	1.4	4.9	0
	1:10	2.0	5.7	0
	1:50	2.4	5.6	10
	1:250	2.9	6.3	5
Aichi	1:2	1.9	4.1	0
	1:10	2.2	6.1	7.5
	1:50	2.4	5.6	10
	1:250	3.0	6.7	20
B		3.2	6.9	42.5

* dilution of seed virus (log_{10}) which infects 50% of test group by aerosol.

** EID_{50}, log_{10}; geometric mean of individual titrations of 5 animals in each group.

† extent of lung lesions (%); 5 animals in each group.

the dilution of seed virus) which infected 50% of the animals. Furthermore, although the difference is small, X-31 immunization appeared to increase resistance more than Aichi virus immunization.

4) Pulmonary virus titers 3 days after challenge were reduced most in animals given the largest doses of either vaccine, but animals given smaller doses of X-31 vaccine were more resistant than mice given equivalent doses of Aichi vaccine.

5) The most sensitive index of increased resistance following immunization was the 7 day lesion score. Thus, vaccine groups which lacked serum antibody, and were not more resistant to the initiation of infection (MID_{50}) and did not have appreciably lower pulmonary virus titers 3 days after infection, nevertheless had less extensive pneumonia than control animals 7 days after infection. On the other hand, susceptibility to the initiation of infection, as measured by the MID_{50} provided the most accurate index of vaccine dose.

Antigenicity of Aichi and X-31 vaccine in rabbits

19 rabbits were injected intravenously with graded dilutions of Aichi and X-31 vaccine as shown in Table 7. Bleedings were obtained at 0, 10, 40 and 48 days after injection, with a booster given on day 40. Hemagglutination inhibition (HI) tests were performed with Aichi, X-15 and X-15(HK) viruses using the microtiter technique with RDE-treated sera and human O RBC. In this experiment, the minimal antigenic dose or AD_{50} of neither vaccine was defined, as HI antibody to Aichi virus was demonstrated in all animals. However, a dose response relationship was observable with the lower potency Aichi vaccine as is illustrated in Fig. 1. This figure demonstrates as well the lesser response to the neuraminidase antigens as measured by X-15(HK) - H_{equi_1} N_{hk} virus, which measures antibody only to the neuraminidase of the HK virus. Clearly, higher dilutions of vaccine virus able to stimulate antihemagglutinin were inadequate to stimulate the production of antineuraminidase.

The correlation of HI and PI (plaque inhibiting) antibody is illustrated in Table 8, which summarizes data from rabbits immunized with the Aichi vaccine. In the plaque inhibition tests, the antisera were incorporated into the agar overlay as previously described (Jahiel and Kilbourne, J. Bact., 1966, 92:1521). In general, a concordance of HI and PI was seen with a factor of 10 separating the two systems.

Plaque size reduction (PSR) was compared with HI versus X-15(HK) and EI as other measures of antineuraminidase. PSR was equivalent in sensitivity with direct inhibition of enzymatic hydrolysis (EI). (Table 8)

TABLE 7

HEMAGGLUTINATION-INHIBITING ANTIBODY TITERS IN SERA OF RABBITS IMMUNIZED WITH FIVE FOLD DILUTIONS OF AICHI AND X-31 VIRUS VACCINES

		ANTIGEN								
		Aichi			X-15			X-15(HK)		
		Day			Day			Day		
Vaccine		10	40	47	10	40	47	10	40	47
Aichi	1:2	5	7*	10	0	1	3	0	2	4
	1:2	7	7	13	0	2	4	0	3	4
	1:10	7	5	12	0	1	3	0	2	3
	1:50	4	3	7	0	0	0	0	0	0
	1:50	3	4	7	0	0	0	0	0	0
	1:250	2	1	4	0	0	0	0	0	0
	1:250	2	0	3	0	0	0	0	0	0
X-31	1:2	5	9	13	0	2	4	0	4	5
	1:2	5	6	13	0	1	4	1	2	5
	1:2	6	6	15	0	2	3	0	4	4
	1:10	6	6	14	0	0	3	0	3	4
	1:10	4	6	14	0	1	4	0	3	3
	1:10	4	7	11	0	0	4	0	4	2
	1:50	2	4	9	0	0	3	0	0	3
	1:50	2	2	6	0	0	1	0	0	0
	1:50	4	7	8	0	1	2	0	3	3
	1:250	2	3	6	0	0	0	0	0	0
	1:250	1	4	6	0	0	2	0	0	1
	1:250	1	3	7	0	0	1	0	0	0

* reciprocal of serum dilution ($\log_2$) inhibiting hemagglutination; initial dilution - 1:10

(Booster injection identical with initial injection given on day 40.)

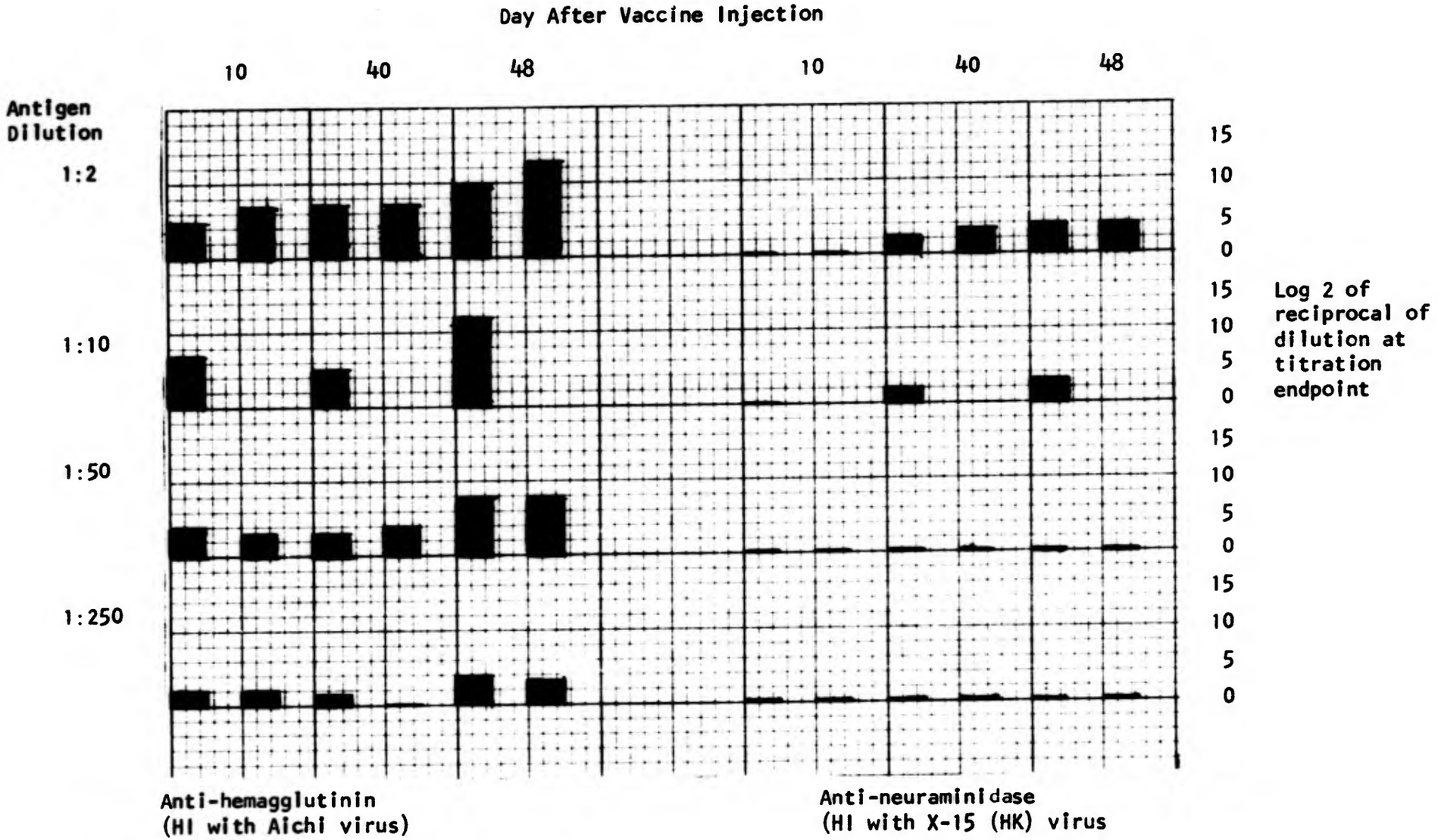


Fig. 1. Synthesis of antibody to Influenza virus (Aichi) envelope proteins after injection of rabbits with varying doses of Aichi vaccine. (Data from table 7). Booster injection given on day 40.

Table 8

Correlation of Hemagglutination-Inhibiting and plaque inhibiting antibody response in Rabbits injected with graded doses of Aichi vaccine

		Titers of 48 day antisera				
rabbit	antigen dilution*	$\frac{HI}{Aichi}$ [a]	$\frac{PI}{Aichi}$ [b]	$\frac{HI}{X\text{-}15\overline{(HK)}}$ [c]	$\frac{PSR}{A_0\,E\overline{(HK)}}$ [c]	$\frac{EI^+}{A_0\,E\overline{(HK)}}$ [c]
403	1:2	5120	51200	80	6400	76
404	1:2	40960	25600	80	25600	80
405	1:10	20480	25600	40	6400	81
407	1:50	640	6400	<10	1600	40
408	1:50	640	6400	<10	800	2
409	1:250	80	800	<10	<400	<1
401	1:250	40	800	<10	<400	<1

* 1:2 dilution (1.0 ml) = 225 CCA units

a measure of antihemagglutinin

b measure of neutralizing antibody

c measure of antineuraminidase

\+ EI = *in vitro* inhibition of viral enzymatic hydrolysis (release of n-acetyl neuraminic acid)

N.B. - 0 day HI titers were all <10; 0 day PI and PSR titers were <400; 0 day EI titers were <1.0.

Attempts to distinguish between plaque inhibition of virus containing both HK antigens (HK/Aichi) and inhibition of HKe, which contained only the hemagglutinin of the HK virus, were confounded (Table 9) by an unprecedented failure of antihemagglutinin antibody to cause PI with this virus. Rather, PSR - heretofore characteristic only of anti-neuraminidase antibody - was observed. This phenomenon is now under study. Preliminary experiments demonstrate that PSR of HKe is induced by normal rabbit serum gamma globulin which conceivably may shield the virus from neutralization with anti HA.

A remarkable boost in PSR but not PI titer after the 40 day injection (Table 9; Fig. 2) suggests that a maximal response to hemagglutinin but not to the viral enzyme had been achieved earlier. In Fig. 2 are shown also another measure of antineuraminidase response (EI) compared with antihemagglutinin (HI) and PI response in a single rabbit given the maximal dose of Aichi vaccine.

Preliminary antigenicity studies in man

550 CCA units of X-31 vaccine in 1 ml. doseage as a single subcutaneous injection was administered to 16 subjects associated with the Department of Microbiology at the Mount Sinai School of Medicine. Thirteen subjects were adults, ranging in age from 22 to 49, three were children, two of whom were 14 years of age and the other 11. Eight subjects were male and eight were female. One subject (JM) had had known, laboratory documented, Hong Kong influenza in 1968. The others were not known to have had Hong Kong influenza. Whole blood for serum was taken just prior to vaccination and 28 days later for study of specific antibody response to the vaccine. The results of this pilot investigation are presented in detail in Table 10, which provides a direct comparison of 9 serologic tests involving 6 viruses and 3 techniques for the measurement of antineuraminidase and antihemagglutinin antibodies.

The apparently inferior response to the neuraminidase antigen suggested by the EI results, may have been colored by the unavailability for study in EI titers of paired sera from good HI responders (in 5 instances). The failure of PSR to demonstrate significant response in most subjects, contrasts with its equivalent sensitivity to the EI test manifest in mice and rabbits. In contrast to the observations in rabbits, X-15 proved superior to X-15HK in demonstrating antineuraminidase, perhaps because the human subjects had been primed by early experience to the A_2 enzyme contained in X-15 or perhaps because of high non-specific levels of inhibitor to X-15(HK) in base line human sera.

The virtual failure of HKe as an indicator of significant antigenic response in PI tests, but its equivalence with Aichi in HI determinations is notable. This observation probably relates to the

TABLE 9

Appearance of Plaque-Inhibiting (PI) and Plaque size reducing (PSR) Antibody Following Immunization of Rabbits

Antiserum	Day*	Immunogen: dilution	PI TITER Aichi (LP)**	"PSR"++ HKe	PSR TITER $A_0E(HK)$	PSR TITER $A_0E(A_2)$+
R-410	10	X-31 (1:2)	3,200	12,800	<400	<400
	40		25,600	25,600	1,600	400
	48		25,600	204,800	25,600	800
R-404	10	Aichi (1:2)	3,200	1,600	<400	<400
	40	" "	12,800	51,200	800	<400
	48	" "	25,600	204,800	25,600	800

* day after iv injection of antigen; booster of same dilution given day 40

** large plaque (LP) variant of HK/Aichi

\+ X-7

++ Plaque size reduction (PSR) mediated through HA antigen or normal serum

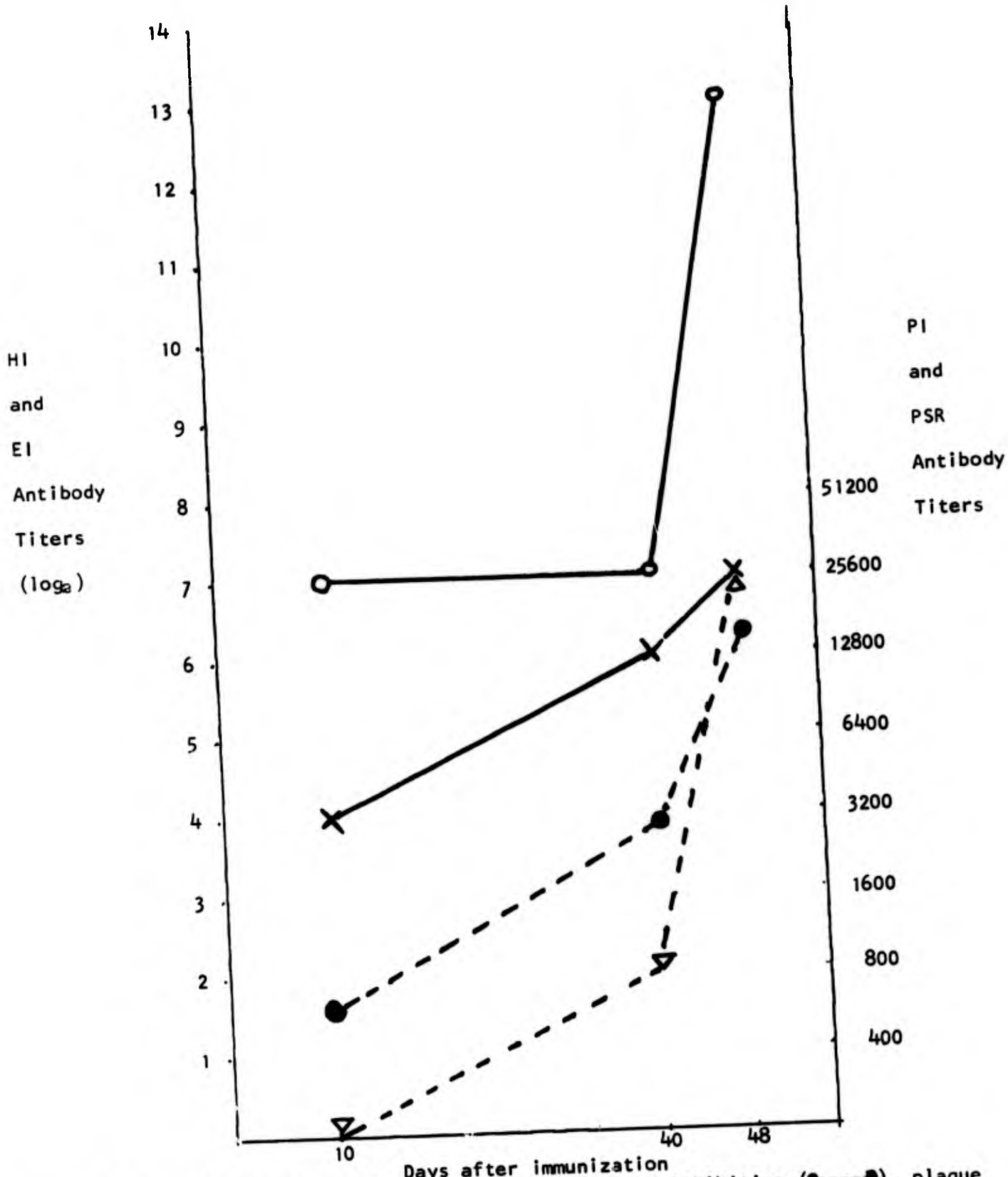


Fig. 2 - hemagglutinating inhibiting (O——O), enzyme inhibiting (●----●), plaque inhibiting (X——X) and plaque size reducing (▽-----▽) antibody titers following immunization of a rabbit with a 1:2 dilution of Aichi vaccine (booster dose given on day 40)

Table 10

Measurement by 9 Serologic Tests of Antibody response to X-31 vaccine in man

Subject	Serum Date	anti-neuraminidase PSR $A_0E(HK)$	PSR $A_0E(A_2)+$	HI X-15(HK)	HI X-15	EEI $A_0E(HK)$	anti-HA PI HKe	PI HKE(HK)**	HI HKe	HI HKE(HK)**
EDK	12/23	<400	400	<10	10	<1.0	<400	<400	<10	<10
	1/20	600	800	<10	40	9.8	<400	800	10	10
LL	12/23	<400	400	-	-		-	800	<10	<10
	1/21	800	800	-	-		-	3200	20	20
KL	12/23	<400	800	80	10	<1.0	-	800	10	<10
	1/20	400	800	160	10	7.0	-	>3200	640	640
JM	12/25	800	800	<10	160	-	-	3200	40	40
	1/20	800	400	10	40	16.0	-	6400	80	80
PSw	12/31	<400	<400	10	10	-	-	400	-	<10
	1/21	400	<400	10	20	-	-	2400	-	160
VH	12/23	<400	400	20	10	<1.0	<400	<400	<10	<10
	1/20	400	800	40	80	8.0	<400	3200	20	20
JR	12/23	400	400	320	<10	<1.0	<400	<400	<10	<10
	1/20	400	400*	320	40	11.2	<400	1600	10	20
DB	12/23	800	800	40	80	7.3	400	3200	160	80
	1/21	800	800*	40	80	18.5	800	6400	320	320
JLS	12/23	800	1600	640	<10	1.4	<400	<400	<10	<10
	1/20	1600	1600	640	80	7.5	<400	1600	10	10
JSw	12/23	<400	800	80	10	1.2	<400	<400	<10	<10
	1/20	<400	800	80	40	3.6	<400	800	10	10
BP	12/23	800	800	80	10	1.0	<400	<400	<10	<10
	1/20	800	800*	160	40	2.0	<400	1600	<10	<10
MR	1/8	<400	800	20	40	-	<400	1600	10	10
	1/22	1600	<1600	40	320	-	*1600	>1600	1280	1280
ESw	12/31			10	160					160
	1/21			10	80					640
LSw	12/31			10	40				40	40
	1/21			10	160				160	160
CSw	12/31	<400	800	10	40		<400	800	20	20
	1/21	<400	1600	10	40		3200	3200	5120	5120

Table 10 (cont.)

Subject	Serum Date	anti-neuraminidase PSR A_0E(HK)	anti-neuraminidase PSR $A_0E(A_0)$+	anti-neuraminidase HI X-15(HK)	anti-neuraminidase HI X-15	EI A_0E(HK)	anti-HA PI HKe	anti-HA PI HKE(HK)**	anti-HA HI HKe	anti-HA HI HKE(HK)**
GS	12/23	400	400	20	<10	<1.0	<400	400	10	<10
	1/21	400	800	20	10	2.0	<400	1600	20	20
4X rises/ total no.		2/14	1/14	0/15	8/15	5/9	2/10	11/14	6/14	11/16

* >PSR

** Aichi

\+ X-7

Summary of Exps. 2192 and 2195

peculiar refractoriness of this virus to neutralization as shown also in the rabbit antibody studies.

In summary, all but two of 16 subjects (87%) showed significant response to the vaccine by at least one test. Of these two subjects (both of whom showed a two-fold increase in plaque inhibiting antibody to Aichi virus) one had had previous Hong Kong influenza and both had high initial levels of specific antibodies as determined by all tests.

Prevention of experimental influenza in volunteers (Baylor Study)

44 volunteers (approximately 15 per group) were injected with either the X-31 or Aichi vaccine or with a gelatin-saline control (Eli Lilly & Co. Lot 2MY67) on November 8, 1969. Challenge with 1,000 $TCID_{50}$ of the Texas Baylor HK strain in first human embryo kidney passage was carried out 28 days later on December 6, 1969. Blood for serologic analysis was obtained on November 8, December 6 and January 3, 1970. Serologic response is summarized in Table 11. All vaccinees had significant neutralizing antibody response to either vaccine. Responses to the two vaccines were comparable. Significant mean antibody increase after challenge was observed only in the control group.

Clinical response to challenge infection in vaccinated and control groups (Table 12)

Following challenge, virus was isolated from 11 out of 14 control subjects, and 6 in the control group became ill. Of the 15 subjects given the Aichi vaccine, 5 shed virus and 1 became ill, while only 2 of those immunized with the X-31 vaccine shed virus and no illness was noted in this group.

Baylor Study

Table 11.

Summary - Mean Serum Neutralizing and Antineuraminidase Antibody Titers Following Vaccination and Infection with A2/Aichi/2/68 Influenza Virus

Neutralizing Antibody

	Vaccination 11/8	Challenge+ 12/6	Rise 11/8-12/6	1/3	Rise 12/6-1/3
Control	--	< 1*	--	5.00	4.86 (< .001)**
A2/Aichi/2/68	< 1	6.27	6.07 (< .001)	7.80	1.53 (< .02)
A2/HK/31/68	< 1	8.00	7.64 (< .001)	8.57	0.57 (> .20)

Antineuraminidase Antibody

	11/8	12/6	Rise 11/8-12/6	1/3	Rise 12/6-1/3
Control	1.97	2.47	0.50 (> .20)	4.04	1.57 (< .01)
A2/Aichi/2/68	1.89	5.72	3.83 (< .001)	5.55	-.16 (> .20)
A2/HK/31/68	2.32	6.19	3.87 (< .001)	6.19	-.004 (> .20)

* Titers expressed as $\log_2$ of the reciprocal of the serum dilution.

** p value

\+ 1000 $TCID_{50}$/0.5 ml of Texas - Baylor HK strain - 1st HEK passage.

Baylor Study

Table 12.

RESPONSE TO CHALLENGE INFECTION

WITH A2/AICHI/2/68 INFLUENZA VIRUS

Group	No. of Subjects	Virus Isolation		Clinical Illness	
		No.	p	No.	p.
Control	14	11	--	6	--
A2/Aichi/2/68	15	5	.036*	1	.06
A2/HK/31/68	14	2	.004	0	.02

* Fisher Exact Test

(data of Drs. Couch and Douglas)

(Submitted by Dr. David Fedson)

Baylor X-31 Vaccine Study: Summary of Antineuraminidase Antibody Responses to Vaccination and Challenge Infection with A2/Aichi/2/68 Influenza Virus.

Methods:

Antineuraminidase antibody (ANAb) was measured by enzyme inhibition using minor modifications of the technique of Laver and Kilbourne (Virology 30: 493, 1966). Virus and antibody were incubated overnight at 4°C before adding fetuin substrate, and then incubated at 37°C for one hour. Serial four-fold dilutions of antiserum were used and all determinations done at least twice. ANAb titers were calculated by computer. ANAb titers reported are not the means of the two separate determinations. They are the titers determined when each of the subjects sera were assayed together in the same test. The availability of separate determinations done at other times provided useful information on the reproducibility of the assay. Duplicate titers were within ± 0.6 $\log_2$ in 75% of the determinations and within ± .75 $\log_2$ in 90% of the determinations. For the values eventually reported, the error of the method is now considerably smaller.

Results:

1. Antibody Response to Vaccination. In both the A2/Aichi/2/68 (Aichi) and A2/HK/31/68 (X-31) vaccinated groups, all subjects experienced a four-fold or greater rise in neutralizing antibody. The mean rise in the X-31 group (7.64) was significantly greater than the rise in the Aichi group (6.07, $p < .05$). Regarding ANAb, 12/15 in the Aichi group and 13/14 in the X-31 group had a four-fold or greater rise in titer. The mean rises in both groups were similar ($p > .20$).

2. Control Group Antibody Response to Infection, and Comparison to Vaccination Groups. Neutralizing antibody responses were four-fold or greater in 11/14 of the control subjects following infection. The remaining three subjects failed to shed virus or show rises in neutralizing antibody and ANAb. They probably escaped infection. Considering the control group as a whole, the mean rise in neutralizing antibody (4.86) was less than that in the Aichi group (6.07) but the p value was .20. It was significantly less than the neutralizing antibody rise in the X-31 group (7.64, $p < .01$). In comparing antibody responses following infection and vaccination it is more reasonable to include in the control infection group only those subjects (11) who were truly infected. Using only these 11 subjects, the mean rise in neutralizing antibody was 6.00. This was similar to the rise of 6.07 in the Aichi group ($p > .20$), but less than that in the X-31 group ($p \cong .05$). Regarding ANAb there were no significant differences between the mean titers of ANAb in these groups before infection or vaccination. In the control group, 6/14 showed a four-fold or greater increase in ANAb following infection. The mean ANAb rise in the 14 control subjects was less than four-fold (1.57) and this was significantly less than the mean rise in both the Aichi (3.83, $p < .01$) and X-31 (3.87, $p < .001$) groups. If only the 11 subjects in the control group who experienced infection are compared to the two vaccination groups, the mean ANAb rise (1.94) was still

less than four-fold and again was significantly less than the mean rises in the Aichi ($p < .05$) and X-31 ($p < .01$) groups. It was not expected that the ANAb rise following infection would be lower than the rise following vaccination. Mean initial ANAb titers were similar, and the distribution of the initial ANAb titers did not seem to bias the resulting ANAb rises. This can be seen by inspecting the accompanying figure which shows the ANAb response to infection and vaccination. Titers are expressed as $\log_2$ of the reciprocal of the serum dilution. It is evident that in only a few instances was the ANAb rise following infection greater than the rise following vaccination when initial titers were closely similar. Previous experience in both man and experimental animals has suggested that the ANAb rise following infection is greater than the rise following vaccination. Why these data suggest the reverse is not clear. Perhaps one reason might be an unusual level of antigenicity of the neuraminidase in both vaccines.

3. Relationship between Initial Titer of ANAb and ANAb Rise Following Infection and Vaccination. For this analysis, the three control group subjects who failed to shed virus are excluded from the analysis. In the remaining 11 subjects there was a strong negative correlation between the initial ANAb titer and the ANAb rise 28 days following infection ($r = -.8091$, $p < .005$). In other words, those subjects with low initial titers had higher rises in ANAb than did those with higher initial titers. The same negative correlation was also seen in the Aichi ($r = -.8001$, $p < .0005$) and the X-31 ($r = -.6615$, $p < .005$) groups.

4. Vaccine-Induced Protection Against Challenge Infection. The number of subjects who shed virus was significantly reduced in the Aichi (5/15, $p = .036$) and the X-31 (2/14, $p = .004$) groups when compared to virus shedding in 11/14 subjects in the control group. Clinical illness was also reduced in both vaccine groups (Aichi, $p = .06$; X-31, $p = .02$).

5. Relationship between Virus Shedding of Clinical Illness and Serum Antibody Levels in the Control Group. The control group as a whole lacked neutralizing antibody before challenge infection (2/14 had titers of 1:2, but they will be regarded as lacking antibody for this analysis). It was therefore possible to examine the effects of virus shedding or illness on the rise in both neutralizing and antineuraminidase antibody. The effects of initial titers in ANAb on the severity of clinical illness and duration of virus shedding were also determined. This analysis involved determinations of Spearman rank correlation coefficients using all 14 individuals in the control group. The results were as follows:

a. Isolation/Illness $r = +0.150$, $t = 0.526$, $p > .20$
The duration of virus shedding was in no way correlated with the severity of clinical illness.

b. Isolation/Neutralizing Ab Rise $r = +0.712$, $t = 3.512$, $p < .005$
Illness/Neutralizing Ab Rise $r = +0.416$, $t = 1.583$, $p \cong .071$

There was a highly significant correlation between the duration of virus shedding and the subsequent rise in neutralizing antibody. Individuals who shed virus for longer periods had higher rises in neutralizing antibody. There was also a definite trend toward higher neutralizing antibody titers in those with more severe clinical disease, but in this case the correlation failed to reach significance at the .05 level.

c. Illness/ANAb Rise $r = +0.544$, $t = 2.246$, $p < .025$
Isolation/ANAb Rise $r = +0.403$, $t = 1.526$, $p \cong .076$

Those subjects with more severe clinical illness had higher rises in ANAb and there was also a tendency for a longer period of virus shedding to be followed by a higher rise in ANAb.

d. Neutralizing Ab Rise/ANAb Rise $r = +0.681$, $t = 3.225$, $p = < .005$

There was an excellent correlation between rises in both antibodies. Those individuals with a high rise in neutralizing antibody also showed a high rise in ANAb.

e. Illness/ANAb-Day 0 $r = -0.205$, $t = 0.725$, $p > .20$

Although initial impressions suggested that persons with lower levels of ANAb prior to infection were those who experienced more severe clinical disease, this impression was not sustained despite repeated assays of Day 0 ANAb titers.

f. Isolation/ANAb-Day 0 $r = -0.314$, $t = 1.147$, $p \cong .118$

There was a definite trend which suggested that virus was shed for longer periods of time in those persons who had lower levels of ANAb prior to infection, although this negative correlation did not reach a statistically significant level. This trend was confirmed in a further assay in which all 14 day 0 specimens were assayed in the same enzyme inhibition test ($r = -0.362$, $t = 1.348$, $p \cong .106$). Furthermore, when the three patients who failed to shed both virus and develop antibody responses were excluded from the analysis, the trend remained ($r = -0.435$, $t = 1.450$, $p = .091$; for all specimens assayed on the same day, $r = -0.426$, $t = 1.413$, $p = .096$).

Serum Neutralizing and Antineuraminidase Antibody

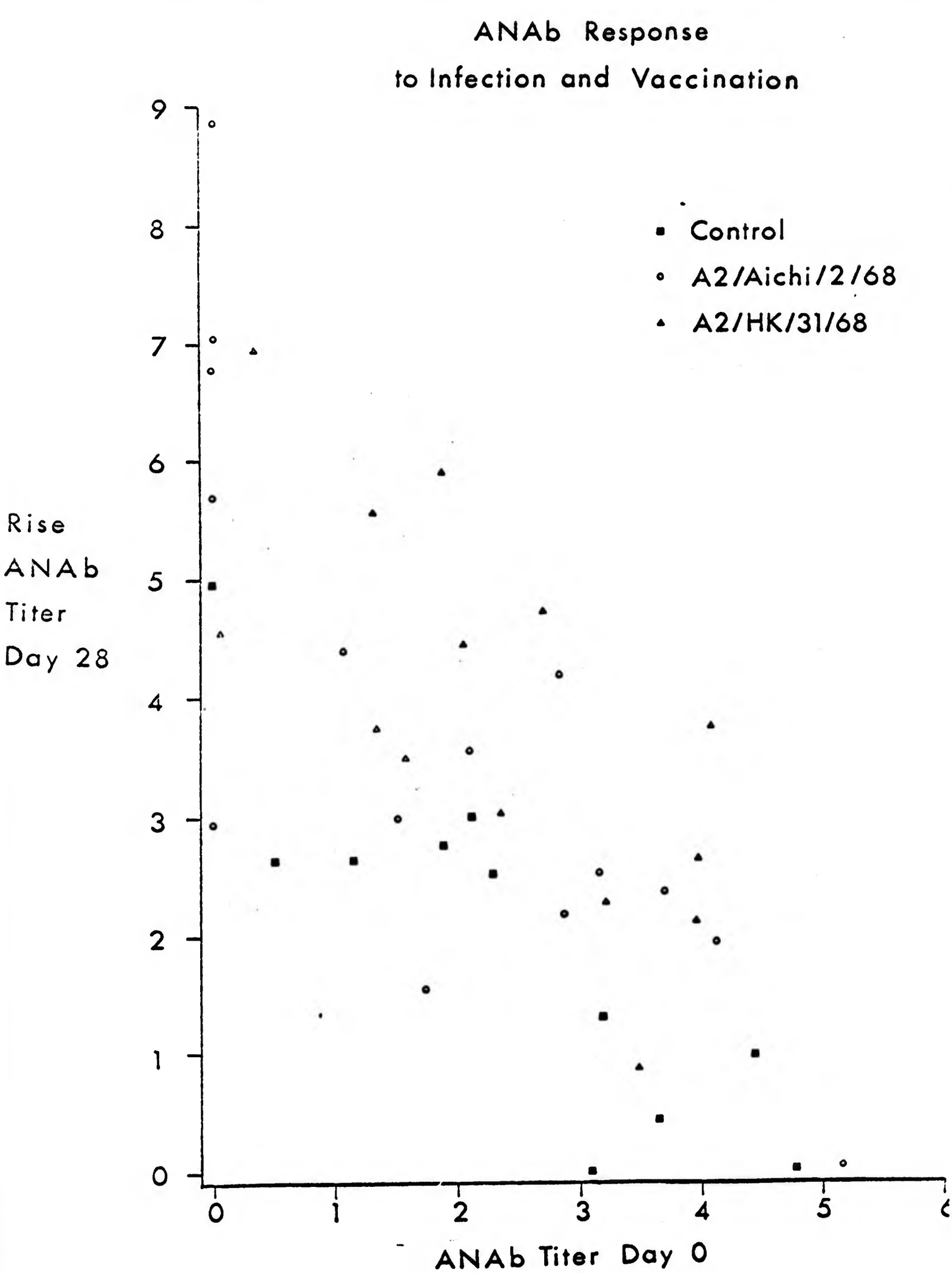
Response to Infection or Vaccination

	No. of Subjects	Neutralizing Antibody			Antineuraminidase Antibody		
		Day 0	Rise Day 28		Day 0	Rise Day 28	
Control Infection	11*	< 1**	6.00 (±.69)	≅ .05†	2.46 (±.46)	1.94 (±.46)	<.05
A2/Aichi/2/68 Vaccination	15	< 1	6.07		1.89	3.83	
A2/HK/31/68 Vaccination	14	< 1	7.64		2.32	3.87	

* Only 11 subjects of 14 in control group who shed virus are included.

** Titers expressed as $\log_2$ of the reciprocal of the serum dilution.

† p value. Except as noted in brackets, all other comparisons between groups were not significant, with p values $\geq .20$.


ANAb Response
to Infection and Vaccination
Control
A2/Aichi/2/68
A2/HK/31/68
Rise
ANAb
Titer
Day 28
9
8
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6
5
4
3
2
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0
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3
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5
ANAb Titer Day 0

(From report submitted by LTC. Raymond Coultrip)

Prevention of natural infection with X-31 vaccine (Ft. Ord Study) - Preliminary Report

Field Design

The study population consisted of newly inducted recruits who received at least six weeks of basic combat training (BCT) at Fort Ord. Personnel arriving at Fort Ord for their basic training are initially processed at US Army Recrption Station, where they remain for approximately three days before assignment to a training company. While at the Reception Station, the recruit undergoes a medical processing and receives the first in a series of scheduled immunizations. Each week the input of recruits from the Recrption Station is divided among five training companies being formed. Normally a recruit is assigned to a company for nine weeks; the first week is called "fill week", followed by eight weeks of basic training, graduates receive advanced training of various types, and at various Army Training Centers. Thus, the cohort (the company) is dissolved upon completion of basic training and the trainees are lost to the study. The design of the field study, therefore, was tailored to meet these requirements.

Individuals entered the study on day 3 at Reception Station, at which time they completed medical processing, and immediately prior to assignment to a training unit. At that time they were separated into two groups, vaccinees and controls, by a process of random selection using the last non-zero digit of their social security number. Written informed consent was obtained from the vaccinee group prior to administration of the experimental vaccine. The standard Army-issue influenza vaccine, usually given on day 3 at Reception Station, was withheld from all recruits in the study until completion of basic training.

The first recruits entered the study on 30 January 1970. All the receptees subsequently processed through the Reception Station through 3 April comprised the study population. A total of 50 companies took part in the study. Since a second (14-day convalescent) blood specimen could not be obtained from men hospitalized in their last two weeks of basic training, the attack rates pertain only to recruits observed over a six-week period of training. Trainees who left the study before completing six weeks of training (discharge, AWOL, transfer off-Post) were excluded from the study and subsequent analysis. The drop-out rate was less than 1 percent.

Antigenicity Studies

The first two complete companies formed after 30 January 1970 (A-2-1 and A-4-2) were selected for the antigenicity studies. Antibody response was determined by serial bleedings taken immediately before, and three and seven weeks after vaccination. One-third of the personnel in the two companies received one ml (556 CCA units) of experimental vaccine; the remainder served as controls and received no influenza

vaccine. All sera were tested for hemagglutination-inhibition (HI) and complement fixing (CF) antibodies.* Vaccine response versus natural infection was determined by the quality and quantity of antibody response. Whereas natural infection normally produces sharp rises in titer for both HI and CF antibodies in susceptible individuals, influenza vaccine ordinarily produces only HI antibody elevation with little, if any, CF antibody response. A four-fold or greater rise in HI antibody titer following vaccination was indicative of vaccine seroconversion.

Those judged to have experienced a natural infection which produced the antibody response were excluded from the analysis of vaccine induced antibody response. This method of selection, though fraught with certain interpretive errors, was thought to portray a more accurate indication of vaccine-induced antibody response than inclusion of persons with natural infection. Personnel comprising A-4-2 entered the study on 3 February 1970, at which time the first blood specimen was taken, and the experimental vaccine was administered to the vaccine.group. Subsequent bleedings of A-4-2 were on 26 February and 25 March 1970. Personnel designated for A-2-1 entered the study on 30 January and 3 February; therefore, part of the blood collection and immunizations of the vaccine group occurred on each of the two days. The second and third bleedings of A-2-1 occurred on 25 February and 23 March 1970, respectively.

Efficacy (Protection) Studies

Forty-eight companies comprised the efficacy study. The objective was to evaluate the protective efficacy of the experimental vaccine against overt and subclinical influenza A disease by intensive laboratory surveillance of all study-group personnel hospitalized for acute respiratory disease (ARD), and by serial serological testing of selected companies for subclinical disease. The experimental vaccine was administered to every fifth recruit passing through the Reception Station. All hospitalized ARD patients from the study group had an acute blood specimen and throat swabs taken for virus isolation within 24 hours of hospital admission. The swabs were immediately placed into Charcoal Viral Transport Media and maintained at 5 degrees (C) until transported to the Sixth US Army Medical Laboratory. A convalescent blood specimen was taken in 14 days. This phase of the study was combined with the ARD surveillance program for basic trainees already in existence at Fort Ord. All the sera were tested for CF antibodies against the soluble antigens of influenza A, influenza B, adenovirus (RI-67 adenovirus type 4), and Mycoplasma pneumoniae. A four-fold or greater rise in CF antibody titer was indicative of disease or inapparent infection.

*HI antigens: A2/Aichi/2/68, A2/Aichi DBS, X-31, X-15, X-15HK, HK_E, PR8
CF antigens: a2/Aichi/2/68

Fort Ord data

Table 13

Immunological response of BCTs as measured by
Various Parent and Hybrid Influenza Antigens after vaccination with
the X-31 influenza hybrid and/or natural infection

Status	Number of Men	CF[a]	4-fold or greater rise in titer by the HAI test[b]						
			Aichi 2/68[b]	Aichi D8S	X-31	X-15	X15HK	HKe	PR8
Vaccinated[c]	116								
a. 23 days	105	6 (6%)	75 (71%)	72 (69%)	78 (74%)	27 (26%)	39 (37%)	65 (62%)	2 (2%)
b. 51 days	115	8 (7%)	71 (62%)	73 (64%)	80 (70%)	30 (26%)	42 (36%)	63 (55%)	3 (3%)
Controls[d]	227								
a. 23 days	212	6 (3%)	10 (5%)	11 (5%)	11 (5%)	4 (2%)	13 (6%)	16 (8%)	3 (1%)
b. 51 days	221	28 (13%)	26 (11%)	33 (15%)	31 (14%)	18 (8%)	29 (13%)	28 (13%)	5 (2%)

a. CF Test: Soluble Aichi/2/68 antigen prepared from the CAM of 12 day old chicken embryos. Seroconversion indicates possible natural infection.

b. HAI: Hemagglutination-inhibition test using "V" antigen prepared from the allantoic fluid of 12 day old chick embryos.

c. Recruits received 1 ml influenza X-31 vaccine containing 550 CCA units intramuscularly. See Antigenicity Study in text.

d. Controls received no treatment and were members of the same Companies as the vaccinees. See Antigenicity Study in text.

Table 14

Hemagglutination-Inhibition (HI) and Complement Fixation (CF) Antibody Response of Vaccinees and Controls. Two Companies of Basic Trainees.*

		Geometric Mean Titer					
		Bleeding 1 (before vaccination)		Bleeding 2 3 weeks later		Bleeding 3 7 weeks later	
	Number	HI	CF	HI	CF	HI	CF
Vaccinees	101	27.9	8.1	121.4	12.5	107.3	9.19
Controls	173	29.9	7.7	33.7	7.93	26.6	7.27

*Individuals experiencing natural infection were excluded.

Table 15

Hospitalized Cases of Influenza A in Study Group

		Flu A		No. Flu A	
	Number	Number	%	Number	%
Vaccinees	1,682	6	0.36	1,676	99.64
Controls	7,934	104	1.3	7,830	98.7
Total	9,616	110	1.1	9,506	98.9

$X^2 = 10.1$

$P = < .002$ (1df)

Four companies (in addition to the two antigenicity companies) were selected for subclinical infection monitoring. Two companies were selected in March (B-5-1 and B-4-3), and two companies were selected in April (A-5-2 and H-4-3). The experimental vaccine was administered on Day 2 in the Reception Station. The first blood collection was taken four to ten days after arrival at Fort Ord. The second and third blood specimens were collected in the third and seventh week of training.

The sera from the four subclinical companies were tested for CF antibodies against soluble Aichi/2/68 antigen. A four-fold or greater rise in antibody titer was interpreted as influenza infection.

The attack rates for the vaccinees and control were determined, and a comparison was made between the two groups for statistical significance using the Chi square $(X)^2$ test.

The immunologic response of recruits as measured by hemagglutination inhibition tests with 7 different viruses is illustrated in Table 13. HK/Aichi and X-31 viruses were comparable in detecting significant response in 70 to 74 percent of the vaccines. HKe virus (which contains only the hemagglutinin of the HK virus) was somewhat less sensitive, while the HK neuraminidase-containing X-15 and X-15HK viruses detected response in 1/4 to 1/3 of the subjects.

Table 14 presents the HI and CF responses of vaccinees and controls. It is notable that the initial HI titer was approximately 3 times that of the corresponding CF titer and there was no significant difference between the initial titer of vaccinees and control. Three weeks later there was a sharp rise in the HI titer of the vaccinees, whereas the HI titer of the controls remained relatively constant. At 7 weeks the HI titer of vaccinees decreased slightly but continued to remain at a high level. The CF titer, in contrast, rose only slightly in the vaccinee group.

Efficacy study

Fort Ord experienced a moderate outbreak of influenza A during the study period, reaching a peak incidence in mid-March. Among a group of 9,616 trainees, there were 110 hospitalized cases of influenza A for an overall attack rate of 1.1%. Among the 1,682 vaccinees, there were 6 hospitalized cases of influenza A. One case was diagnosed by viral isolation plus positive serology and 5 cases were diagnosed by serology alone. There were 104 hospitalized cases of influenza A among the 7,934 controls, as illustrated in Table 15.

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13. ABSTRACT

A recombinant influenza virus X-31 which is antigenically identical with the new Hong Kong (HK) variants but which grows to high yields in chick embryos, has been produced by hybridization of the standard Aichi vaccine strain with A_0/PR8 virus. With this virus, purified inactivated vaccine was produced by Evans Medical Ltd. in England for clinical trials in the United States under the auspices of the Commission on Influenza.

Correlative and comprehensive studies of the X-31 vaccine and standard HK/Aichi vaccine of matched CCA activity have been conducted in mice, rabbits and man in 6 different laboratories. These studies demonstrated:

1) Significant protection of volunteers from experimental influenza was afforded by both vaccines as determined by reduction in illness and shedding of virus after challenge. 2) The efficacy of X-31 vaccine in preventing natural infection at Fort Ord; 104 hospitalized cases of influenza were found among the 7,934 controls (1.3%), while only 6 (0.36%) occurred in the 1,682 vaccinees ($p=<0.002$). 3) That immunogenicity in experimental animals correlated well with antigenicity and protection data in man. It was possible to distinguish between the two vaccines on the basis of their relative effects on human and mouse ID_{50} and their capacity to stimulate the production of hemagglutination-inhibiting, neutralizing and plaque-inhibiting antibody.

The differing immunogenicity of the two vaccines was not predictable from their CCA concentrations, which did not differ significantly.

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