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PATHOGENICITY AND IMMUNOGENICITY OF VARIOUS STRAINS OF INFLUENZA VIRUS FOR MICE*

KANZEN NAKAMURA

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Osaka
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SUMMARY The pathogenicity and immunogenicity of 13 strains of influenza virus and one strain of HVJ (parainfluenza 1 virus) were tested in mice. Several mice in each group were made to inhale serial ten fold dilutions of infected allantoic fluid for 10 minutes. Studies were made on their death, lung consolidation and antibody response. It was found that influenza virus strains could be classified into 4 groups. Group 1, consisting only one of the 13 strains, showed neither pathogenicity nor immunogenicity. Group 2, consisting of 5 strains, caused an antibody response in mice without pathogenicity and group 3, consisting of three strains, was pathogenic only when inoculated in large doses. Group 4, consisting of 5 strains, was pathogenic even when given in a small dose.

INTRODUCTION

During investigations on live influenza vaccines, various problems were proposed to be discussed between Soviet and Japanese scientists. (NAKAMURA, 1961 a, b; NAKAMURA, 1964; NAKAMURA and OKUNO, 1961; OKUNO, 1963; OKUNO and NAKAMURA, 1964; OKUNO *et al.*, 1960). Soviet workers claim that 1) many egg passaged strains loose not only their pathogenicity but also their immunogenicity, and so that they cannot be used as vaccinal strains. 2) Strains which are difficult to reisolate can not give a good antibody response and 3) the inhalation method can not be used for vaccination because this method inevitably causes febrile reactions (RITOVA and EVSTIGNEEVA, 1960; SMORODINTSEV, 1957). These opinions are all contradictory to our observations.

To solve these problems and to obtain more effective live influenza vaccine, further fundamental experiments with animals seemed necessary. Mice were chosen as the experimental animals and used for studies on the immunogenicity and pathogenicity of various influenza virus strains. The most important character for a vaccinal strain is a high immunogenicity with a low pathogenicity. Thus it was necessary to devise a method for measuring both the immunogenicity and the pathogenicity of influenza virus strains against mice and an indicator was found of the suitability of the strains for live influenza vaccine in mice.

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MATERIALS AND METHODS

1. *Virus materials*

Infected allantoic fluids were centrifuged at low speed and the supernatants were used as virus materials. The influenza virus strains used in this study are shown in Table 1.

2. *Inhalation of virus materials*

Kano's method, which is a modification of Loosli's method (Loosli *et al.*, 1943) was used for inhalations of influenza virus by mice.

A nebulizer was connected with a desiccator with a side arm containing the mice and the air was passed from an air compressor to the nebulizer at a rate of 6 liter per minute. The virus materials were sprayed in at a concentration of 0.02–0.03 ml per liter of air. Serial ten fold dilutions of virus material in broth were administered to groups of 5 mice for 10 minute periods. The air from the desiccator was conducted into the iodine tincture to inactivate the influenza virus in the exhaust air. The same nebulizer was used throughout this work.

3. *Observation of mice*

The survival of animals was checked every day and 2 weeks after inhalation surviving animals were bled and the extent of consolidation of their lungs was examined. Individual sera were stored at −15°C until their antibody titers were determined.

4. *Hemagglutination inhibition (HI) test*

Sera were heated at 56°C for 30 minutes unless otherwise mentioned. Volumes of 0.25 ml of serial two fold dilutions of sera were prepared and then 4 units of hemagglutinin in 0.25 ml saline were added. The virus-serum mixtures were mixed with 0.5 ml of 0.5 per cent chicken rbc. Titers were expressed as the reciprocals of the highest dilution in which hemagglutination was completely inhibited.

5. *Complement fixation (CF) test*

A modification of Kolmer's method was used. Supernatant fluid after high speed centrifugation (20,000 rpm for 60 min.) of homogenized infected

TABLE 1 *Influenza virus strains and materials used*

Virus strain	Passage history	Virus material	
		HAU/ml (log_2)	EID_{50}/0.1 ml (log)
A/SW15[a]	M38E31	10	7.3
A/PR8/34[b]	F148M596E73	11	8.9
A/Weiss/44[c]	F3E8	9	8.5
A1/FM1/47[d]	E14M53E4M9E1	11	8.5
〃	〃	19	10.25
A1/Omachi/1/53[e]	E7M21E130	10	8.25
〃	E7M21E131	15	9.5
〃	E8	8	8.0
A2/Okuda/57[f]	E276	10	8.25
A2/Arakawa/57[g]	E26	9	8.0
A2/Adachi/2/57[h]	E4M17E72	11	8.7
B/Lee/40[i]	F8M296E66	12	8.5
B/Setagaya/3/56[j]	E116	10	7.7
〃	E9M21E9	11	6.8
B/Taiwan/4/62[k]	E32	9	7.75
HVJ (Parainfluenza 1)/Z/52[l]	Several hundred egg passages	12	9.5

a), c), d), e), g), h) and j) were kindly given by Dr. H. Fukumi of the Institute of National Health. b) and i) were provided by Mr. T. Yamaguchi of the Osaka Microbial Diseases Research Foundation. f) and l) were isolated in our laboratory.

allantoic membrane was used as S antigen. Virus suspension purified by high speed centrifugation of infected allantoic fluid was used as V antigen.

Serial two fold dilutions of sera were prepared in 0.2 ml volumes of PBS and then 2 units of complement and 2 units of antigen in 0.1 ml PBS were added. After incubation at 37°C for an hour, 0.1 ml of 3 per cent sensitized bovine rbc was added.

After reincubation for one hour at 37°C with frequent shaking, the titers were read as the reciprocals of the highest dilution which showed 50 per cent or less hemolysis.

6. *Titration of neutralizing (NT) antibody*

Serial two fold dilutions of sera were prepared in 0.4 ml of broth and then 0.4 ml of 100 EID_{50}/0.1 ml of influenza virus was added. After one hour's incubation at 37°C, 0.2 ml samples of each dilution was inoculated into three eggs.

The antibody titer was expressed as the highest serum dilution which caused neutralization of influenza virus in over 50 per cent of the eggs examined after a two or three day incubation period at 36°C.

7. *Reactivation of heated antibody with serum cofactor*

Cofactor (STYK, 1961) in the form of native mouse serum was added at a concentration of 1 : 80 (HI) or 1 : 10 (CF, NT) and reactivation (STYK *et al.*, 1958) of heated antibody was examined.

RESULTS

First, the immunogenicity and pathogenicity of strain PR8 was tested. Serial ten fold dilutions of virus material containing $10^{8.9}$ EID_{50}/0.1 ml were sprayed into the desiccator containing groups of five mice. As shown in Table 2, all the mice died in the groups which inhaled material diluted from 10^{0} to 10^{-2}. The five mice survived for 4 to 5 (mean 4.4) days with the material diluted 10^{0}, 5–6 days (mean 4. 2) with material diluted 10^{-1} and 6–8 (mean 6.6) days with material diluted 10^{-2}. Survival increased with dilution of the virus material. In the group which inhaled material diluted 10^{-3}, three mice died within 9 to 12 days and two survived for 14 days although they had severe lung consolidation at the end of this period. These two mice showed a remarkable antibody response. No animals died with material diluted 10^{-4}. All mice had slight lung consolidation and a high antibody response. No animals died with a dilution of 10^{-5}. Of these animals one showed both an antibody response without any consolidation, while three showed neither an antibody response nor pathological changes. With 10^{-6} dilution, neither an antibody response, nor lung consolidation was observed. Indicators such as MLD_{50}, CSD_{50} (50 per cent consolidation dose), ABD_{50} (50 per cent antibody response dose) and EID_{50} can be calculated. In this experiment, $MLD_{50} = 10^{3.1}$, $CSD_{50} = 10^{4.7}$ and $ABD_{50} = 10^{4.9}$. MLD_{50} and CSD_{50} were relatively high and the value of ABD_{50}/MLD_{50} was $10^{1.8}$ which is relatively low. This strain proved to be fairly virulent but not so virulent as to kill all animals inoculated with a dilution of 10^{-4} or with more diluted material. The dose range in which death and lung consolidation did not occur but in which there was an antibody response was very narrow.

The immunogenicity and pathogenicity of various strains of influenza virus were also tested by the same method. The results obtained were as follows.

TABLE 2 *Immunogenicity and pathogenicity of influenza A, PR8 strain in mice*

Dilution of virus material[a] (−log)	Day of death or extent of lung consolidation[b]	HI antibody titer[c] (log_2)
0	4 4 4 5 5	
1	5 5 5 5 6	
2	6 6 6 7 8	
3	9 9 12 ### ##	10 10
4	+ + + + +	10 9 9 10 10
5	+ − − − −	9 10 0 0 0
6	− − − − −	0 0 0 0 0
Control	− − − − −	0 0 0 0 0

a) PR8 : F148M596E73. 2048HAU/ml. $10^{8.9}$ EID_{50}/0.1 ml. b) The numbers show the survival days. +, ##, ###, ###, indicate pulmonary consolidation in 25, 50, 75 and 100 per cent of lung, respectively, at the end of obserbation period. c) 0=<5.

TABLE 3 *Immunogenicity and pathogenicity in mice of only egg passaged Omachi strain of influenza A1 virus*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)
0	− − − − −	0 0 0 0 0
1	− − − − −	0 0 0 0 0
2	− − − − −	0 0 0 0 0
3	− − − − −	0 0 0 0 0
4	− − − − −	0 0 0 0 0
5	− − − − −	0 0 0 0 0
6	− − − − −	0 0 0 0 0
Control	− − − − −	0 0 0 0 0

TABLE 5 *HI antibody response of the Okuda strain measured by erythrocytes of various animals*

Dilution of virus material (−log)	HI antibody titer (log_2) by erythrocytes of			
	Chick	Guinea pig	Human	Mouse
0	0	6	8	10
1	0	6	8	9
2	0	6	8	9
3	0	0	6	7
4	0	0	0	0
5	0	0	0	0
Control	0	0	0	0

TABLE 4 *Immunogenicity and pathogenicity of influenza A2, Okuda strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)	
		With native sera	With heated sera
0	− − − − −	9 9 8 9	0 0 0 0 0
1	− − − − −	8 8 9 8 7	0 0 0 0 0
2	− − − − −	7 0 7 0 7	0 0 0 5 0
3	− − − − −	6 0 0 0 0	0 0 0 0 0
4	− − − − −	0 8 0 0 0	0 0 0 0 0
5	− − − − −	0 0 0 0 0	0 0 0 0 0
6	− − − − −	0 0 0 0 0	0 0 0 0 0
Control	− − − − −	0 0 0 0 0	0 0 0 0 0

Omachi strain of influenza Al virus passaged only in eggs: Neither pathogenicity nor an antibody response was observed even at the highest dose of virus (Table 3).

Okuda strain of influenza A2 virus: No pathogenicity was observed. An antibody response was observed only with native sera and no HI antibody was observed with inactivated sera (Table 4). However an HI antibody response was observed even with heated sera when the reaction time was prolonged, or an antigen was employed which seemed more sensitive to antibody, or when erythrocytes of other animals such as guinea pigs humans or mice were used (Table 5). Heated antisera to the Adachi and Arakawa strains showed no HI antibody titer with Okuda strain under ordinary conditions.

Arakawa strain: No pathogenicity but an antibody response was observed and with unheated sera more highly diluted virus material provoked an antibody response (Table 6).

A2, Adachi, B, Setagaya and B, Taiwan strain: No pathogenicity but an antibody re-

TABLE 6 *Immunogenicity and pathogenicity of influenza A2, Arakawa strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)	
		Native sera	Heated sera
0	– – – – –	10 10 9 7 7	8 8 9 6 5
1	– – – – –	9 10 10 9 9	9 7 7 6 8
2	– – – – –	7 9 9 9 8	5 8 6 7 7
3	– – – – –	8 8 7 10 9	6 0 0 6 7
4	– – – – –	0 9 0 7 0	0 0 0 0 0
5	– – – – –	0 0 0 0 0	0 0 0 0 0
6	– – – – –	0 0 0 0 0	0 0 0 0 0
Control	– – – – –	0 0 0 0 0	0 0 0 0 0

TABLE 7 *Immunogenicity and pathogenicity of influenza A2, Adachi strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (−log_2)
0	– – – – –	10 10 10 10 9
1	– – – – –	10 10 8 7 10
2	– – – – –	9 10 10 8 8
3	– – – – –	7 8 10 8 11
4	– – – – –	8 10 8 0 8
5	– – – – –	0 0 0 0 0
6	– – – – –	8 0 0 0 0
Control	– – – – –	0 0 0 0 0

TABLE 8 *Immunogenicity and pathogenicity of influenza B, Setagaya strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)
0	– – – – –	10 11 12 12 9
1	– – – – –	11 12 12 12 12
2	– – – – –	12 11 11 12 10
3	– – – – –	12 11 10 9 12
4	– – – – –	8 0 0 0 0
5	– – – – –	0 0 0 0
6	– – – – –	0 0 0 0 0
Control	– – – – –	0 0 0 0 0

sponse was observed. No great differences were found between these three strains (Tables 7-9).

Parainfluenza 1 (HVJ) Z strain: Pulmonary consolidation was observed only with undiluted virus material which did not cause death of the mice. An HI antibody response was observed only with native sera and hemagglutination inhibition was somewhat incomplete. With human erythrocytes, complete inhibition of hemagglutionation was observed even with heated sera although with a very low antibody titer (Table 10).

TABLE 9 *Immunogenicity and pathogenicity of influenza B, Taiwan strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (−log_2)
0	– – – – –	8 8 9 8 7
1	– – – – –	9 8 9 5 9
2	– – – – –	9 8 9 8
3	– – – – –	0 6 0 0
4	– – – – –	7 0 0 0 0
5	– – – – –	0 0 0 0 0
Control	– – – – –	0 0 0 0 0

Mouse passaged Omachi and Setagaya strains: Pathogenicity was observed only with a high dose of these viruses (Tables 11, 12). The survival period of mice was very long and was more than 7 days even in the group which inhaled highly concentrated virus (Table 13). An antibody response was observed with a fairly low dose of virus. Thus the range in which an antibody response was obtained without any pathogenic reaction was fairly wide.

A, Weiss strain: Death of mice was observed only with a high dose of virus and the survival period of mice was more than 7 days. Lung consolidation was observed with a relatively low dose of the virus. The antibody response was generally acompanied by pathogenic changes (Table 14).

B, Lee, SW 15. A, PR8. and A1, FM1 strains: These strains were highly pathogenic and few strains showed an antibody response without any pathogenic changes (Tables 2, 15–17). Death of mice occurred with a low dose of virus. The

TABLE 10 *Immunogenicity and pathogenicity of parainfluenza type 1 (HVJ), Z strain in mice*

Dilution of virus material (log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)																	
		Chick erythrocytes										Human erythrocytes							
		Native sera					Heated sera					Native sera					Heated sera				
0	### ## + – ###	7	8	8	7	7	0	0	0	0	0	7	8	8	8	8	5	5	5	5	5
1	– – – – –	8	7	8	7	7	0	0	0	0	0	7	6	6	6	6	5	5	6	5	5
2	– – – – –	7	7	7	7	–	0	0	0	0	0	7	7	7	7	–	0	5	6	5	–
3	– – – – –	7	7	7	6	6	0	0	0	0	0	8	7	8	7	7	5	5	5	0	0
4	– – – – –	6	7	7	7	7	0	0	0	0	0	7	7	8	7	7	0	0	5	0	5
5	– – – – –	0	0	0	0	0	0	0	0	0	0	7	6	5	5	5	0	0	0	0	0
6	– – – – –	0	0	0	0	0	0	0	0	0	0	5	5	5	5	5	0	0	0	0	0
Control	– – – – –	0	0	0	0	0	0	0	0	0	0	5	5	5	5	5	0	0	0	0	0

TABLE 11 *Immunogenicity and pathogenicity of mouse passaged Setagaya strain of influenza B virus in mice*

Dilution of virus material (–log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)				
0	9 9 ## – ##			9	9	8
1	– – – – –	10		8	8	8
2	– – – – –	9	8	9	8	7
3	– – – – –	6	8	6	7	7
4	– – – – –	0	0	7	0	0
5	– – – – –	0	0	0	0	0
6	– – – – –	0	0	0	0	0
Control	– – – – –	0	0	0	0	0

TABLE 12 *Immunogenicity and pathogenicity of influenza A1, Omachi strain in mice*

Dilution of virus material (–log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)				
0	11 12 13 ## ###				8	8
1	+ – – – –	8	9	9	8	7
2	– – – – –	8	8	9	7	9
3	– – – – –	8	10	10	7	8
4	– – – – –	0	8	0	0	8
5	– – – – –	0	0	0	0	0
6	– – – – –	0	0	0	0	0
Control	– – – – –	0	0	0	0	0

survival period was very short with a high dose of virus. With the Lee strain, the survival period was longer, and with the SW15 strain it was slightly longer with a high dose of virus than with FM1 or PR8. With the PR8 strain, the lower the viral dose, the less was the extent of pulmonary consolidation. This phenomenon was observed constantly with the PR8 strain, but never with the FM1 strain. With a larger dose, the survival period was shorter. However the survival period was never shorter

TABLE 13 *Immunogenicity and pathogenicity of concentrated influenza A1, Omachi strain*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)
0	5 7 8 9 9	
1	8 9 10 11 11	
2	8 9 11 + ⅲ	7 9
3	+ + + − −	10 8 9 8 9
4	− − − − −	9 7 8 6 8
5	− − − − −	0 5 9 8 8
6	− − − − −	0 0 0 0 0
7	− − − − −	0 0 0 0 0
8	− − − − −	0 0 0 0 0
Control	− − − − −	0 0 0 0 0

TABLE 14 *Immunogenicity and pathogenicity of influenza A1, Weiss strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)
0	7 8 9 9 9	
1	9 11 + ⅲ ⅲ	11 10 9
2	+ ⅱ ⅱ + +	10 12 10 10 9
3	9 − − ⅱ ⅲ	10 10 12 12
4	− − − − −	0 0 0 9 5
5	− − − − −	0 0 0 0 0
6	− − − − −	0 0 0 0 0
Control	− − − − −	0 0 0 0 0

TABLE 15 *Immunogenicity and pathogenicity of influenza B, Lee strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)
0	6 7 7 8 8	
1	7 9 9 9 9	
2	8 9 9 10 11	
3	ⅲ ⅲ ⅱ ⅱ +	10 8 8 8 7
4	+ − − − −	8 10 0 8
5	− − − − −	0 0 0 0 0
6	− − − − −	0 0 0 0 0
Control	− − − − −	0 0 0 0 0

TABLE 16 *Immunogenicity and pathogenicity of Swine influenza SW15 strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)
0	4 4 7 7 8	
1	6 7 7 7 7	
2	7 7 8 9 9	
3	9 9 11 11 14	
4	9 − + ⅲ ⅱ	0 8 8 8
5	− − − − −	0 0 0 0 0
6	− − − − −	0 0 0 0 0
Control	− − − − −	0 0 0 0 0

TABLE 17 *Immunogenicity and pathogenicity of influenza A1, FM1 strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation	HI antibody titer (log_2)
0	3 3 4 4 6	
1	4 4 5 5 6	
2	5 6 7 7 7	
3	8 10 ⅲ ⅲ ⅲ	11 12 10
4	ⅱ ⅱ ⅲ + ⅲ	11 12 11 10 12
5	− ⅱ ⅲ ⅱ −	0 11 10 11 8
6	− − − − −	0 0 0 10 0
Control	− − − − −	0 0 0 0 0

TABLE 18 *Immunogenicity and pathogenicity of concentrated influenza A, FM1 strain in mice*

Dilution of virus material (−log)	Day of death or extent of lung consolidation					HI antibody titer (log_2)				
0	3	3	4	4	4					
1	3	3	4	4	4					
2	3	4	5	5	6					
3	6	6	6	6	7					
4	7	7	7	8	8					
5	8	10	++	+++	+++			10	8	10
6	12	+	++	−	+		9	10	8	8
7	−	−	++	−	−	0	0	9	0	0
8	−	−	−	−	−	0	0	0	0	0
Control	−	−	−	−	−	0	0	0	0	0

than 3–4 days even with the highest dose of FM1 strain (Table 18).

These strains can be calssified into the following four groups.

(1) Strain with neither immunogenicity nor pathogenicity
(2) Strains with immunogenicity without pathogenicity
(3) Strains with immunogenicity and slight pathogenicity
(4) Strains with immunogenicity and high pathogenicity

The strains examined are classified as shown in Fig. 1. All the strains examined, except one, showed an antibody response in not only HI but also in CFV, CFS and NT antibodies. Reactivation of HI antibody with cofactor was observed in some strains but in others it could not be tested because of the inhibitor sensitivity of the virus strains. A phenomenon which seemed to resemble " reactivation ", was also observed in CFV and CFS antibodies but not in neutralizing antibody in these conditions (Table 19).

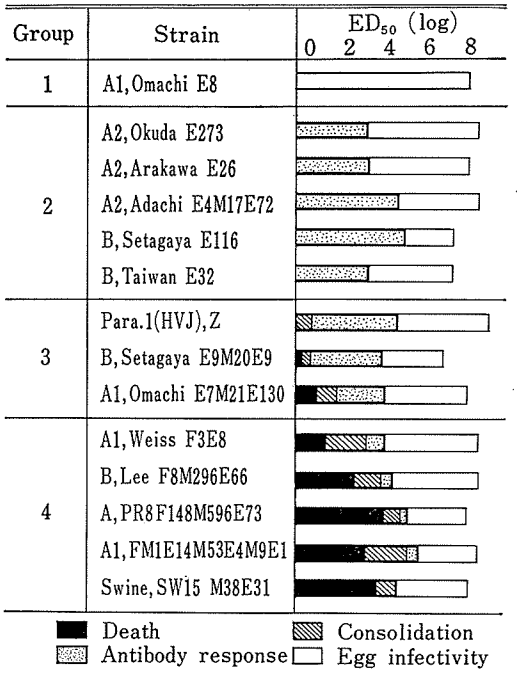

FIGURE 1 Immunogenicity and pathogenicity of various strains of influenza virus in mice

DISCUSSION

1. *Methods for measuring the virulence of influenza virus against mice*

HORSFALL (1939) used the 50 per cent mortality end point and the 50 per cent maximal lesion score end point for titration of influenza virus, although not for measuring virulence. FADEEVA (1953) used the mortality rate to study the virulence of different strains of influenza virus. DAVENPORT (1954) used the lung lesion score to distinguish minute differences in the virulence of influenza viruses. In these experiments, they used an intranasal inoculation method after light etherizing of mice. These methods were sometimes disturbed by irregular mortality and lung consolidation, which could be largely avoided by the use of the inhalation method.

So far, no one has tried to measure virulence in relation to the antibody response. With the present approach, it has become possible to distinguish minute differences in virulence of different strains of influenza virus, as discussed in the next section.

TABLE 19 *HI, CF and NT antibody response of mice with various strains of influenza virus*

Material for inhalation		Serum	Antibody titer (log_2)							
			HI		CFV		CFS		NT	
Strains and titers	Dilution (−log)		Pre	Post	Pre	Post	Pre	Post	Pre	Post
A, Omachi E8		Native	7	7	2	2			1>	2>
1024 HAU/ml	0	Heated	4>	4>	1>	1>			1>	2>
$10^{8.0}EID_{50}$/0.1 ml		Heated+NMS	Not measured		1>	1>			1>	2>
A2, Okuda E276		Native	4>	8	2	4	1	4	0>	6
1024 HAU/ml	0	Heated	4>	4	1>	1>	1>	1>	1>	6
$10^{8.75}EID_{50}$/0.1 ml		Heated+NMS	4>	8	1>	3	1>	3	1>	7
B, Setagaya E116		Native	8	10			2	5	1>	
1024 HAU/ml	0	Heated	4>	9			1>	3	1>	6
$10^{8.5}EID_{50}$/0.1 ml		Heated+NMS	Not measured				1>	5	1>	7
A2, Adachi E4M17E72		Native	4>	9	1	4	1	3	1>	
2048 HAU/ml	1	Heated	4>	7	1>	1	1>	1>	1>	8
$10^{8.5}EID_{50}$/0.1 ml		Heated+NMS	4>	9	1>	4	1>	3	1>	8
A1, Omachi E7M21E130		Native	7	10	2	5	2	6	1>	8
2048 HAU/ml	2	Heated	4>	8	1>	2	1>	2	1>	10
$10^{8.5}EID_{50}$/0.1 ml		Heated+NMS	Not measured		1>	6	1>	5	1>	10
A1, FMI F14M53E13		Native	8	11	2	5	2	5		
2048 HAU/ml	2	Heated	4	9	1>	2	1>	2		
$10^{9.0}EID_{50}$/0.1 ml		Heated+NMS	Not measured		1>	6	1>	5		
A, PR8 F148M596E83		Native	4>	11	2	5	2	5	1>	9
2048 HAU/ml	4	Heated	4>	9	1>	2	1>	2	1>	8
$10^{7.9}EID_{50}$/0.1 ml		Heated+NMS	4>	11	1>	5	1>	5		

NMS: Normal mouse serum

2. *Titration of the immunological and pathological properties of influenza virus strains in mice*

The immunological and pathological properties of virus can be schematized in Fig. 2. With a very small dose, no response will occur. With a higher dose, pulmonary consolidation will occur. With further stimulation, death will occur after severe pulmonary consolidation. The biological values of ABD_{50} (50 per cent antibody response dose), CSD_{50} (50 per cent consolidation dose), MLD_{50} (LD_{50} in mice) and EID_{50} (ID_{50} in eggs) can be used to indicate

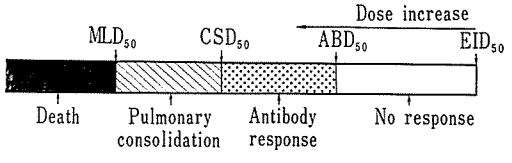

FIGURE 2 Scheme of immunogenicity and pathogenicity of influenza virus in mice.

numerically the immunogenicity and pathogenicity of the strains. The lower the value of ABD_{50}/MLD_{50} or ABD_{50}/CSD_{50}, EID_{50}/MLD_{50} and EID_{50}/CSD_{50}, the higher the virulence. The value of ABD_{50}/EID_{50} of a strain will show the immunogenicity or susceptibility of the strain to mice.

Various other observations also seem to be useful. One strain, FM1, provoked severe consolidation even in a very low dose at which infection barely occured. However another strain, PR8, provoked moderate or no consolidation at a minimum dose, while a severe consolidation was observed only with a higher dose. The phenomenon that the greater the dose, the more severe is the consolidation, was observed not with the former but with the latter strains. This seems to indicate the difference in the pathogenicity of these two strains. The survival period must also be considered, especially with the maximum dose. The higher the dose, the shorter the survival period, and the survival period finally becomes constant. With less virulent strains, the survival period is never as short as with highly virulent strains. The minimum survival period was 3–4 days in FM1 strain and 7–9 days in the Omachi, Weiss and Lee strains.

3. *Relation between the dose and virulence or the antibody response*

In testing whether or not a strain is virulent, the virus dose seems to have been rather neglected. It is bovious that some strains are virulent only at a high dose. Some strains caused lung consolidation at lower dose, while death was observed only with higher dose. In the present experiments, not even the highly virulent strains were virulent enough to cause death of animals at a munimum dose. Cosequently, virulence cannot be examined without consideration of the viral dose.

A large dose of one strain seems to be needed and only a small dose of another strain is required for an antibody response. This seems to indicate that the dosage must be considered in reference to the antibody response. With highly susceptible strains only very few virus particles are needed to evoke an antibody response. Less susceptible strains require numerous virus particles. Non susceptible strains are quantitatively ineffective in causing an antibody response and, no relation was found between the virus dose and the HI antibody reponse.

4. *Prospects of live influenza vaccine*

Two features are important in selecting a strain for use as live influenza vaccine. These are good immunogenicity and weak pathogenicity, which are shown with indicators such as ABD_{50}/MLD_{50} or ABD_{50}/CSD_{50}. The higher the value of the indicator, the more suitable the strain is for use as live influenza vaccine. Strains which are classified in groups 2 and 3 in the present paper can be used for vaccination of mice.

The suitability of the strains for use as live influenza vaccine can be shown numerically by indicators. Such indicators as ABD_{50}/MLD_{50} for humans cannot be measured. Thus some *in vitro* indicator must be devised for mice as a first step in obtaining an indicator for showing the suitability of the strains for live influenza vaccine in humans. Strains which are immunogenic but non-pathogenic for mice are now available and it may be expected that strains which are suitable for humans will also become available. The opinion that there are always hazards connected with the use of live vaccine may be denied at least with respect to use of live influenza vaccine for mice.

5. *Reactivation of heated antibody with serum cofactor*

The Okuda strain of influenza A2 virus showed no antibody response with inactivated sera, which means that the strain provokes only an incomplete or weak antibody. But it does not mean that the strain is especially weak immunogenically. It is understood that the virus has a low susceptibility to antibody, because an antibody response was observed even with heated sera when an antibody-susceptible anti-

gen such as the Arakawa strain, was used. Heated mouse antibody acts very weakly against the Okuda strain in the absence of a cofactor.

The phenomenon, similar to reactivation phenomenon, was observed in the complement fixation test, but further studies are needed on this.

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