INFECTION AND IMMUNITY, Aug. 1973, p. 137-144

Vol. 8, No. 2
Printed in U.S.A.

Antibody Response of Hamsters to A2/Hong Kong Virus Vaccine after Priming by Heterotypic Virus Infection

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Received for publication 16 April 1973

Hamsters previously infected with influenza virus A1/FM/1/47 produced serum hemagglutination inhibition (HI) antibody in response to 1/100 the antigenic dose of inactivated influenza virus A2/Hong Kong vaccine necessary to induce antibody in normal animals. This priming effect was believed to be due to the virus infection which caused an immune response to a virus antigen common to both the infecting virus and the virus vaccine; this antigen acted as a carrier for the specific vaccine virus hemagglutinin and potentiated the immune response to the new antigen. This theory, which has been established in other immune systems, was tested, and the results obtained did not contradict the conditions imposed in the above explanation. Thus, the priming effect could be transferred to normal hamsters by inoculation of spleen cells from virus-infected animals, and the HI antibody response to the virus vaccine was characteristic of a secondary response. The theory also required that the new antigen be coupled to the carrier protein; however, primed hamsters produced serum HI antibody after inoculation with ether-Tween-split virus vaccine, but there was no proof that this vaccine was completely dissociated.

Studies have shown that, unless hamsters were primed by prior infection with an influenza A virus, the animals would not produce serum hemagglutination inhibition (HI) antibody in response to immunization with 150 chick cell agglutination (CCA) units of inactivated A2/Hong Kong/68 vaccine (C. W. Potter, R. Jennings, W. M. Marine, and C. McLaren, Microbios, in press); similar results have been found for ferrets (McLaren et al., manuscript in preparation). Prior infection with any influenza A virus would prime hamsters to respond to A2/Hong Kong vaccine, but priming could not be induced by infection with influenza B or C viruses. Similar results have been found for other influenza virus vaccines; thus, hamsters previously infected with any influenza A virus respond better to subsequent immunization with inactivated A2/Japan/305/57, A1/FM/1/47, and A/PR/8/34 virus vaccines than normal hamsters (R. Jennings and C. W. Potter, Arch. Gesamte Virusforsch., in press).

In the present studies, the HI antibody response to immunization with inactivated and ether-Tween-split A2/Aichi/2/68 vaccine was measured in hamsters previously infected with heterotypic influenza A viruses. The nature of the antibody response to both the infecting virus and to subsequent immunization with vaccine virus was determined by rate-zonal centrifugation in linear sucrose gradients. In addition, the HI antibody response to immunization with inactivated influenza virus A2/Aichi/2/68 vaccine was measured in hamsters previously inoculated with spleen cells, thymus cells, or serum from influenza virus-infected donor animals.

MATERIALS AND METHODS

Influenza viruses A/PR/8/34 (H0N1), A1/FM/1/47 (H1N1), A2/Singapore/1/57 (H2N2), and A2/Hong Kong/1/68 (H3N2) were strains of virus maintained in our laboratory. Virus pools were prepared by allantoic inoculation of 10-day embryonated hen's eggs. After incubation at 35 C for 48 h, the allantoic fluids were harvested and stored at −80 C. The identity of the viruses was confirmed by cross-hemagglutination inhibition tests by using monospecific ferret antisera.

Virus vaccines. Inactivated influenza virus A1/FM/1/47 and A2/Aichi/2/68 vaccines were obtained from W. Marine, Emory University School of Medicine, Atlanta, Ga. Both vaccines were prepared from virus purified by rate-zonal centrifugation and inacti-

vated with Formalin (W. M. Marine and J. E. Thomas, Brit. Postgrad. J., in press); the A2/Aichi/2/68 vaccine contained 1,714 CCA/ml and had a total protein content of 165 μg/ml, and the A1/FM/1/47 vaccine contained 571 CCA/ml and had a protein concentration of 137 μg/ml. A second influenza A2/Aichi/2/68 vaccine, termed A2/Aichi/2/68 vaccine 2, was obtained from W. Hennesson, Behringwerke AG, Marburg; this vaccine contained 300 CCA/ml and was obtained as a Formalin-inactivated virus vaccine and as an ether-Tween-disrupted virus vaccine.

HI tests. Hemagglutination-inhibition tests were carried out by using microtiter apparatus (11). Before testing, sera were treated with cholera filtrate (Burroughs Wellcome Ltd.) for 18 h at 37 C and subsequently heated for 1 h at 56 C. Serum dilutions were incubated for 10 to 15 min with eight hemagglutinating units of virus (50% end-point) before adding fowl erythrocytes. The HI titers were read after the cells had settled at room temperature and were expressed as the highest serum dilution which caused a 50% reduction in virus hemagglutination.

Experimental design. All the studies were carried out in 2- to 3-month-old Syrian hamsters from a single closed, randomly bred colony at the University of Sheffield. Prior to tests, a blood sample (0.5–1.0 ml) was collected from the orbital sinus of each hamster using a finely drawn Pasteur pipette. Groups of hamsters were then infected intranasally and under light ether anesthesia with 0.2 ml of a live influenza virus preparation; the dose of virus used had been shown in preliminary studies to induce serum HI antibody, and contained $10^{5.5}$ to $10^{6.5}$ mean egg infective dose (EID_{50})/ml. Three weeks after virus infection a further blood sample was obtained from each hamster, and the animals were immunized by intramuscular injection of inactivated virus vaccine or split virus vaccine in an 0.5-ml volume. A third blood specimen was collected 3 weeks after immunization. All hamster sera were stored at −20 C.

Rate-zonal centrifugation. Hamster sera were treated with cholera filtrate for 18 h at 37 C, and then heated at 56 C for 1 h. Linear sucrose gradients of approximately 5.0-ml volume were prepared with the aid of a mixing device from 5 and 20% (wt/vol) sucrose (Analar) in phosphate-buffered saline (pH 7.4), and kept for 1 h at 4 C. The sucrose gradients were layered with 0.2 ml of treated hamster serum mixed with 0.02 ml of human serum; the human serum was included to provide immunoglobulin γM and γG markers of known sedimentation coefficients. After centrifugation at 100,000 × *g* for 5 h in a Spinco L preparative centrifuge at 4 C, and using an SW50 swinging-bucket rotor, 14-drop volumes were collected from the gradients through a needle inserted into the base of the centrifuge tubes. Each fraction was titrated for HI antibody and for human immunoglobulins γG and γM.

The human serum used to supply immunoglobulin markers for the rate-zonal centrifugation studies was obtained from a patient with Waldenström macroglobulinemia. The serum contained low levels of HI antibody to influenza viruses, and these were removed by absorption. Samples (10 ml) of influenza viruses A2/Hong Kong/1/68, A1/FM/1/47, and A/PR/8/34 were centrifuged at 100,000 × *g* for 40 min, and the pellets were resuspended in 2.0 ml of the human serum sample. After overnight incubation at 4 C, the sample was centrifuged at 100,000 × *g* for 40 min and the pelleted material was discarded. This procedure removed detectable HI antibody to the viruses used for absorbtion. The sample was further incubated for 4 h at 4 C with packed fowl cells (final concentration 10%) to remove fowl red cell agglutinins. The serum contained 550 mg of γG per 100 ml and 1,700 mg of γM per 100 ml; these levels were sufficiently high that 0.02 ml of the serum added to the layered hamster serum sample gave easily detectable peaks of both immunoglobulins in the fractions collected after centrifugation. The concentration of γM and γG in the samples from the sucrose gradients was determined by single radial immunodiffusion (2) with the specific antisera prepared against the human immunoglobulins (Burroughs Wellcome Ltd.).

Adoptive transfer of serum or lymphocytes. Twenty-one days after intranasal infection of hamsters with influenza virus A2/Hong Kong/1/68 or A1/FM/1/47, the animals were killed, and the blood, spleens, and thymuses were removed. The organs were finely minced, and the tissue was suspended in medium 199 and further broken down by extrusion of the fragments through a syringe without a needle. The suspension was then filtered through sterile cotton gauze to remove the remaining tissue fragments. The resulting cell suspension, almost entirely composed of single cells, was centrifuged at 1,000 × *g* for 10 min, and distilled water was added to the deposited cells for 30 s (3 ml of water per 0.1 ml of sedimented cells) to lyse the red blood cells; the medium was restored to isotonic strength by adding an equal volume of double-strength saline. The cells were washed three times in medium 199 and resuspended in the same medium to a concentration of 5 × 10^6 viable cells/ml and used immediately to inoculate hamsters.

Normal, adult hamsters were each given 100 rads of X irradiation; this treatment reduced the hamster peripheral leukocyte count by 10 to 30%, but the count returned to normal values 7 to 10 days after treatment. Twenty-four hours after irradiation, groups of hamsters were inoculated intraperitoneally with 5 × 10^6 spleen cells or thymus cells. Further groups of hamsters were inoculated by the intraperitoneal route with 0.5 ml of serum collected from hamsters 21 days after influenza virus infection. The following day, a blood sample was collected from each hamster, and the animals were then immunized intramuscularly with influenza virus vaccine. A further blood sample was collected 3 weeks after immunization.

RESULTS

Immunization of hamsters with A2/Aichi/2/68 vaccine following infection with influenza virus A1/FM/1/47. Hamsters were infected intranasally with influenza virus A1/FM/1/47; serum specimens collected 3 weeks after infection showed that all the animals had developed serum HI antibody to the infecting

virus. The hamsters were then divided into groups of three or four and immunized with different concentrations of inactivated influenza virus A2/Aichi/2/68 vaccine. Similar extinction titrations of the vaccine were carried out in normal hamsters. Blood specimens were collected 3 weeks after immunization, and the results of HI antibody tests on these sera are shown in Table 1.

Immunization of normal hamsters with 1,500 CCA of influenza A2/Aichi/2/68 virus vaccine produced serum HI antibody to A2/Hong Kong/68 virus in all four animals at titers of 1:15 to 1:60; however, inoculation with doses of 500 CCA or less of vaccine failed to induce detectable levels of HI antibody (Table 1). In contrast, for hamsters which had been previously infected with influenza virus A1/FM/1/47, immunization with 15 CCA of A2/Aichi/2/68 vaccine produced serum HI antibody at titers of 1:15 to 1:40, but inoculation with 1.5 CCA failed to induce detectable levels of HI antibody. Thus, hamsters previously infected with influenza virus A1/FM/1/47 produced HI antibody in response to immunization with 1/100 the antigenic dose of inactivated influenza virus A2/Aichi/2/68 vaccine necessary to induce antibody in normal hamsters. Immunization with influenza virus A2/Aichi/2/68 vaccine did not significantly alter the serum HI antibody titers to influenza virus A1/FM/1/47.

TABLE 1. *Antigen extinction titration for inactivated A2/Aichi/2/68 vaccine in normal hamsters and in hamsters previously infected with influenza virus A1/FM/1/47*

Dose of A2/Aichi/2/68 vaccine (CCA)	HI antibody response in hamsters			
	Not previously infected with live virus		Previously infected with influenza virus A1/FM/1/47	
	A1/FM/1/47[a]	A2/Hong Kong/1/68[a]	A1/FM/1/47[a]	A2/Hong Kong/1/68[a]
1,500	—[b]	<10-15	NT[c]	NT
	—	<10-30		
	—	<10-60		
	—	<10-60		
500	—	—	NT	NT
	—	—		
	—	—		
	—	—		
150	NT	—	60-60	<10-15
		—	240-120	<10-15
		—	60-120	<10-20
		—	60-60	<10-30
15	NT	NT	240-320	<10-15
			80-240	<10-15
			>320-240	<10-30
			>320-240	<10-40
1.5	NT	NT	160->320	—
			60-60	—
			240-240	—
			120-240	—

[a] HI antibody titer to virus.
[b] —, < 10 - < 10.
[c] NT, Not tested.

Response of hamsters to whole or to split influenza A2/Aichi/2/68 virus vaccines. Groups of hamsters were infected with influenza virus A1/FM/1/47 or A/PR/8/34. Twenty-one days later, when all the hamsters were shown to have developed specific HI antibody to the infecting virus, the animals were inoculated intramuscularly with either 150 CCA of Formalin-inactivated A2/Aichi/2/68 vaccine 2 or with 150 CCA of ether-Tween-split A2/Aichi/2/68 vaccine 2. The same dose of the two vaccines was also used to inoculate normal hamsters. Blood specimens were taken 3 weeks after immunization, and the results of HI tests are shown in Table 2. Hamsters which had not been previously infected with either A1/FM/1/47 or A/PR/8/34 influenza viruses did not produce detectable levels of serum HI antibody in response to inoculation with 150 CCA of either the whole or ether-Tween-treated vaccine. In contrast, hamsters previously infected with live influenza virus A1/FM/1/47 or A/PR/8/34 produced serum HI antibody to titers of 1:15 to 1:30 in response to infection of 150 CCA of either whole or split virus vaccine. Immunization did not significantly alter the titers of serum HI antibody to the initial infecting virus.

Adoptive transfer studies: lymphocyte transfer. Twenty-one days after intranasal infection with influenza virus A1/FM/1/47, hamsters were bled and cell suspensions were prepared from pooled spleens or thymuses; thymus and spleen cells suspensions were also prepared from normal hamsters. Groups of hamsters, which had been X irradiated the previous day, were inoculated intraperitoneally with 5×10^6 spleen cells or thymus cells from infected hamsters or from normal hamsters; serum specimens collected from these animals 24 h after inoculation did not contain detectable serum HI antibody to influenza virus A1/FM/1/47. At this time, some hamsters of each group were inoculated with 150 CCA of inactivated influenza virus A2/Aichi/2/68 virus vaccine and the others were not immunized. A further blood sample was collected from each animal 3 weeks after immunization. The results of serum HI tests are shown in Table 3.

TABLE 2. *HI antibody response of hamsters to whole or ether-Tween-split A2/Aichi/2/68 vaccine after previous infection with heterotypic influenza viruses*

Hamsters previously infected with:	Form of A2/Aichi/2/68 vaccine 2 (150 CCA)	Change in serum HI antibody titer to virus (before and after immunization with A2/Aichi/2/68 vaccine)		
		A2/Hong-Kong/1/68	A1/FM/1/47	A/PR/8/34
A1/FM/1/47	Whole virus	<5–30	320–320	—[a]
		<5–30	240–120	—
		<5–15	320–320	—
	Ether-Tween-split virus	<5–15	320–240	—
		<5–20	320–240	—
		<5–15	120–120	—
A/PR/8/34	Whole virus	<5–15	—	240–120
		<5–15	—	240–160
		<5–20	—	240–240
	Ether-Tween-split virus	<5–10	—	240–160
		<5–15	—	320–240
		<5–10	—	240–240
None	Whole virus	—	—	—
		—	—	—
		—	—	—
	Ether-Tween-split virus	—	—	—
		—	—	—
		—	—	—

[a] —, <10–<10.

TABLE 3. *HI antibody response of hamsters to influenza virus vaccine, after adoptive transfer of spleen cells or serum*

Adoptive transfer (IP[a] inoculation)	Change in serum HI antibody titer to virus			
	After inoculation of 150 CCA of A2/Aichi/2/68		After inoculation of 150 CCA of A1/FM/1/47	
	A2/Aichi/2/68	A1/FM/1/47	A2/Aichi/2/68	A1/FM/1/47
Hamsters previously infected with A1/FM/1/47				
Spleen cells (5 × 10^6)	<5–15	—[b]		
	<5–15	—	NT[c]	NT
	<5–20	—		
Serum (0.5 ml; HI titer 1:320)	—	30–<5	—	30–<5
	—	15–<5	—	30–<5
	—	60–<5	—	15–<5
Normal hamsters				
Spleen cells (5 × 10^6)	—	—		
	—	—	NT	NT
	—	—		
Serum (0.5 ml)	—	—	—	<5–60
	—	—	—	<5–40
	—	—	—	<5–30

[a] IP, Intraperitoneal.
[b] —, <10–<10.
[c] NT, Not tested.

Hamsters which were inoculated with spleen or thymus cells from normal animals did not produce serum HI antibody after subsequent inoculation with 150 CCA of inactivated A2/Aichi/2/68 vaccine. No detectable HI antibody was found in sera from hamsters inoculated with A2/Aichi/2/68 vaccine after adoptive transfer of thymic cells from hamsters previously infected with influenza virus A1/FM/1/47; however, hamsters inoculated with spleen cells from infected animals and subsequently given 150 CCA of A2/Aichi/2/68 vaccine developed serum HI antibody at titers of 1:15 to 1:20 to A2/Hong Kong virus (Table 3). Serum from hamsters inoculated with A2/Aichi/2/68 vaccine after transfer of spleen cells from A1/FM/1/47 infected animals did not contain HI antibody to A1/FM/1/47 virus.

Serum transfer. Sera were collected from hamsters 21 days after intranasal infection with influenza virus A1/FM/1/47; the HI antibody titer of the pooled sera was 1:320 for influenza virus A1/FM/1/47, and no HI antibody was detectable to other influenza viruses. A pool of sera was also prepared from normal hamsters. Groups of hamsters were inoculated intraperitoneally with 0.5 ml of either normal or postinfection sera. The following day, a blood sample was taken from each hamster, and the animals were immunized with 150 CCA of either inactivated influenza virus A2/Aichi/2/68 or A1/FM/1/47 vaccines. A further blood sample was collected 21 days after immunization. The results are shown in Table 3. Hamsters inoculated with normal hamster sera did not produce HI antibody to influenza virus A2/Hong Kong/1/68 after inoculation with 150 CCA of A2/Aichi/2/68 vaccine, but did produce antibody in response to the same dose of A1/FM/1/47 vaccine (Table 3). Extinction titrations of the two vaccines in hamsters has shown that the inactivated A1/FM/1/47 virus vaccine was a more potent antigen than the A2/Aichi/2/68 vaccine (R. Jennings and C. W. Potter, Arch. Gesamte Virusforsch., in press).

Serum specimens collected from hamsters 24 h after intraperitoneal inoculation of serum containing HI antibody to A1/FM/1/47 virus possessed demonstrable HI titers of 1:15 to 1:60. Immunization of these hamsters with 150 CCA of A2/Aichi/2/68 vaccine did not produce demonstrable titers of HI antibody to the vaccine virus, and the passively acquired antibody to influenza virus A1/FM/1/47 was not demonstrable 3 weeks after immunization. In addition, hamsters inoculated with serum containing HI antibody to A1/FM/1/47 virus did not develop serum HI antibody in response to immunization with 150 CCA of inactivated A1/FM/1/47 vaccine. This was probably due to the passively acquired antibody which prevented the HI antibody response to the vaccine and which had disappeared by 21 days after immunization (Table 3); similar results have been reported for rabies vaccine (13).

Rate-zonal centrifugation studies of hamster HI antibody. Groups of hamsters were infected intranasally and under ether anesthesia with influenza virus A1/FM/1/47, A/PR/8/34, or A2/Hong Kong/1/68; blood samples were collected 8 days and 21 days after infection. At this time, the hamsters were inoculated with 150 CCA of inactivated influenza virus A2/Aichi/2/68 vaccine, and further blood samples were taken 8 and 21 days after immunization. The serum specimens were tested for HI antibody and were examined by rate-zonal centrifugation in 5 to 20% linear sucrose gradients to determine the sedimentation coefficients of the antibody.

Figure 1 shows the sedimentation pattern of the HI antibody present in the serum 8 days and 21 days after infection with influenza virus A1/FM/1/47. For the serum specimen taken 8 days after infection, the HI antibody was detected in two peaks; the position of one peak coincided with that of human γM, and the second peak corresponded to human γG (Fig. 1A). The serum HI antibody present at 21 days after virus infection was detected only in a single peak which was coincidental with that of human γG, included in the gradient as a marker (Fig. 1B). This same hamster, previously infected with influenza virus A1/FM/1/47, was immunized with 150 CCA of inactivated A2/Aichi/2/68 vaccine, and serum specimens were collected 8 and 21 days later. These specimens were also studied by rate-zonal centrifugation in linear sucrose gradients, and the results showed that at both times all the detectable HI antibody was present in a single peak corresponding to that of human γG (Fig. 1C and D). Thus, the initial infection gave an antibody response typical of a primary infection, producing both 19*S* and 7*S* HI antibody, while the subsequent response to immunization was typical of a secondary response with only 7*S* HI antibody detected.

Results similar to those shown in Fig. 1 were found in sera from hamsters infected with various heterotypic influenza A viruses and subsequently immunization with inactivated A2/Aichi/2/68 vaccine. These results are shown in Table 4. The serum HI antibody response in hamster sera 8 days after intranasal infection with influenza viruses A1/FM/1/47, A/PR/8/34,

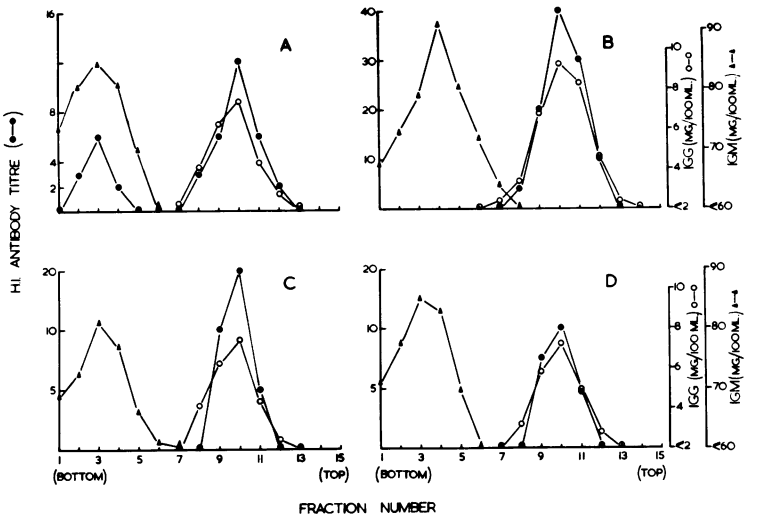

FIG. 1. *Rate-zonal centrifugation of hamster serum HI antibody. Linear sucrose gradients (5–20%) were layered with 0.2 ml of hamster serum together with 0.02 ml of human serum. After centrifugation for 5 h at 100,000 × g, fractions were collected from the base of the gradient through a fraction collector, and tested for HI antibody (●), immunoglobulin G (O), and immunoglobulin M (Δ). A, Serum (diluted 1/3) collected 8 days after infection with influenza virus A1/FM/1/47. B, Serum (diluted 1/3) collected 21 days after infection with influenza virus A1/FM/1/47. C, Serum collected 8 days after immunization with 150 CCA of influenza A2/Aichi/2/68 vaccine. D, Serum collected 21 days after immunization with 150 CCA of influenza A2/Aichi/2/68 vaccine. All serum specimens were from the same hamster; sera A and B were titrated for HI antibody to influenza virus A1/FM/1/47, and sera B and C were titrated for HI antibody to influenza virus A2/Hong Kong/1/68.*

TABLE 4. *HI antibody response to influenza virus infection and subsequent immunization with inactivated A2/Aichi/2/68 virus vaccine*

Virus infection	Hamster no.	Change in HI antibody titer after infection		HI antibody response to immunization with 150 CCA of A2/Aichi/2/68 vaccine	
		8 days PI[a]	21 days PI	8 days PIm[b]	21 days PIm
A1/FM/1/47	1	<5–320 (γG, γM)[c]	640 (γG)	<5–30 (γG)	30 (—)
	2	<5–320 (γM)	320 (γG)	<5–30 (γG)	60 (—)
	3	<5–120 (γG, γM)	320 (γG)	<5–60 (γG)	60 (γG)
A/PR/8/34	1	<5–240 (γG, γM)	320 (γG)	<5–30 (γG)	60 (γG)
	2	<5–240 (γM)	640 (γG)	<5–120 (γG)	120 (—)
	3	<5–320 (γM)	640 (γG)	<5–60 (γG)	60 (—)
A2/HK/1/68	1	<5–60 (γG, γM)	240 (γG)	240–480 (γG)	480 (γG)
	2	<5–15 (—)	60 (γG)	60–1280 (γG)	480 (—)
	3	<5–80 (γG, γM)	120 (γG)	120–640 (γG)	640 (—)

[a] PI, Postinfection.
[b] PIm, Postimmunization.
[c] Change in HI antibody titer (immunoglobulin type of antibody).
[d] —, Not tested for immunoglobulin type.

or A2/Hong Kong/1/68 was in each case found to be either 19*S* or 19*S* and 7*S* antibody. In every case, only 7*S* antibody was found in sera collected 21 days after infection. Rate-zonal centrifugation studies on serum specimens collected from the above hamsters 8 and 21 days after subsequent immunization with 150 CCA A2/Aichi/2/68 vaccine indicated only 7*S* antibody in both serum specimens (Table 4).

DISCUSSION

The present studies show that hamsters primed by prior infection with influenza virus A1/FM/1/47 produced detectable levels of

serum HI antibody in response to 1/100 the antigenic dose of inactivated influenza virus A2/Aichi/2/68 vaccine required to induce HI antibody in normal hamsters. This priming effect is not limited to influenza virus A1/FM/1/47 infection or to A2/Hong Kong vaccine, since infection with a range of influenza A viruses can prime hamsters to respond to a variety of influenza A virus vaccines (R. Jennings and C. W. Potter, Arch. Gesamte Virusforsch., in press; C. W. Potter, R. Jennings, W. M. Marine, and C. McLaren, Microbios, in press). In addition, similar reults have been obtained in ferrets (McLaren et al., manuscript in preparation). The mechanism for the increased responsiveness of primed animals to influenza virus vaccines is not known. However, it is suggested that the exaggerated response to the new virus hemagglutinin was due to the virus hemagglutinin being complexed with carrier antigen of either the whole virus particle or to a portion of the virus hemagglutinin to which the hamster has already responded; thus, the infecting virus and the vaccine virus both contain ribonuclear protein and matrix antigens which are common to all influenza A viruses (9).

The suggested mechanism underlying the increased responsiveness of primed hamsters to inactivated influenza virus vaccines is the same as that proposed to account for observations of other antigen-carrier or hapten-carrier systems. Thus, rabbits or guinea pigs previously immunized with bovine γG show a markedly augmented response to subsequent inoculation of 2,4-dinitrophenol when coupled with bovine γG (1). Similar results have been reported by other workers using other carrier systems (3, 7, 8, 10, 12). From these studies, a number of features of the proposed mechanism have been shown. Thus, the new antigenic determinant must be coupled to the carrier virus particle (8, 12), and secondly the mechanism for the exaggerated response is based on observations which show that the antibody response to immunization of primed animals is a secondary response (8). These two features were tested in the present study.

In the present study, hamsters primed by infection with influenza virus A1/FM/1/47 were able to produce serum HI antibody in response to immunization with either whole virus or ether-Tween-split virus. This result suggested that the response of primed hamsters to influenza vaccine was not dependent on the new hemagglutinin being complexed with carrier antigens. However, it is not known to what extent the split virus vaccine was disrupted; some portion of the virus may remain intact or some reaggregation may take place after ether-Tween treatment. The response of primed hamsters to purified, heterotypic hemagglutinins is now being studied. Rate-zonal centrifugation studies in linear sucrose gradients showed that the antibody response to inactivated influenza virus A2/Aichi/2/68 vaccine in serum collected 8 days after inoculation of primed hamsters was entirely 7*S*. The result was strongly suggestive of a secondary response, since sera collected eight days after primary virus infection contained either 19*S* or 7*S* and 19*S* antibody.

Adoptive transfer experiments showed that inoculation of normal hamsters with lymphocytes from hamsters previously infected with influenza virus A1/FM/1/47 transferred the property of increased responsiveness to A2/Aichi/2/68 vaccine; similar results were obtained for guinea pigs inoculated with hapten-bovine γ-globulin complex following inoculation of lymphoid cells from animals immunized with bovine γ-globulin (6). Passive immunization with serum from primed hamsters did not potentiate the later response to virus vaccine; thus, the presence of humoral antibody to the carrier virus particle was not sufficient to prime animals. The suggested mechanism of the priming effect requires the mutual cooperation of lymphocytes in the response (8, 12); our results further indicate the importance of lymphocytes in the mechanism of primed response.

Although normal hamsters and ferrets do not produce serum HI antibody in response to inoculation with 300 CCA of inactivated influenza virus A2/Aichi/2-68 vaccine, this dose produces a good antibody response in volunteers (4, 5). It remains possible that the response in man is due to prior infection by homotypic or heterotypic influenza A virus and that the response to vaccine is dependent to some degree on a priming infection.

ACKNOWLEDGMENTS

We wish to thank Sir Charles Stuart-Harris and M. G. McEntegart for their advice and criticism, and L. Shepherd and G. Ellis for their excellent technical assistance. These studies were supported by a grant from the Medical Research Council.

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