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*Development of a New Live Attenuated Mumps Virus Vaccine in Human Diploid Cells**

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Abstract. A new live attenuated mumps vaccine was developed in human diploid cells. The S-12 virus was isolated from a 10-year-old girl showing typical symptoms of mumps infection, the diagnosis was confirmed by a pediatrician. The virus was isolated in green monkey kidney cells, without passage in chick embryo cavity or chick embryo fibroblasts. Attenuation of the wild virus was performed by serial passages in human diploid cells (MRC-5). The attenuated virus was characterized by identity tests, as well as by a reduction in plaque size, as marker tests. The virus was free from adventitioue agents and safe for laboratory animals as well as for monkeys. The reactogenicity and immunogenicity of the S-12 virus for man was investigated by administration of a monovalent vaccine to 20 seronegative adult male volunteers and 30 children aged 1 to 5 years without history of mumps infection or vaccination. Seroconversion was obtained in 95% of the vaccinees. The new vaccine has the advantage of not requiring specific pathogen-free eggs, and being free from avian proteins ant therefore can be used in sensitized patients.

Keywords: *Mumps / Viruses / Vaccines / Tissue culture*

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Introduction

Mumps is an acute viral disease of man with frequent complications. infection with mumps virus may cause various forms of central nervous system disorders. Acute neurological sequelae of mumps infections such as deafness, fatal nephritis, pancreatitis, discomforts following parotitis or orchitis and death due to mumps virus are among complications of the infection. To prevent the disease, vaccination of children after their first birthday is highly recommended.

In our country, the imported mumps vaccine has been used ever since the vaccine was available on the world market. Since 1988, the mumps vaccine was produced in our laboratory by using live attenuated Hoshino-L32 strain, granted by Dr S. Makino, Chief, Department of Virology, The Kitasato Institute (Japan). The vaccine is not distributed in this country under the expanded programme of immunization (EPI) for mass use, however, there are increasing demands from pediatricians for mumps vaccine. So far, more than 100000 doses of vaccine have been used locally, either in the form of monovalent mumps vaccine or as a component of the measles, mumps and rubella (MMR) prophylactic. The reactions due to mumps antigen have been reported to be mild, not exceeding 1%, manifested as transient parotitis of short duration. Our intention has been to isolate mumps virus and to attenuate it in human diploid cells (HDC) MRC-5 in order to produce all three components of MMR in HDC, without using chick embryo cells.

Materials and methods

Virus isolation

The virus was isolated from a 10-year-old girl who showed high fever and bilateral parotitis, without any other clinical complications. In the morning, before breakfast, on day 4 after the onset of the disease, the girl had her oral cavity washed with a solution of sucrose. The washing was received in an sterile vial. Equal volumes of 10% bovine albumin, 1000 units of penicillin and 100μg of streptomycin per ml were added. The vial was immersed in ice, transported to the laboratory and was kept at-70°C before use. for virus isolation, the material was thawed and centrifuged for 10 min at 1000 **g**. Supernatant (0.1 ml) was

inoculated directly into eight tubes containing green monkey kidney cells (GMKC) and incubated at 35° C. After 6 days, the specific cytopathic effect (cpe)was observed in one culture; the hemadsorption of guinea-pig red blood cells on day 7 was positive in other tubes of GMKC. The fluid of the tube showing cpe was twice subcultured in GMKC. All cultures showed cpe 5-6 days after incubation at 35°C. The fluid of the third passage in GMKC was pooled and frozen at-70°C for further attenuation.

Viral Strains

The following strains were isolated by Dr K. Sasaki of the Department of Virology at the Kitasato Institute (Japan), and have been used as a reference of wild mumps strains by NIH of Japan. They were kindly supplied by Dr S. Makino.

1. Mumps virus (Sasazaki strain) 3rd passage in Vero cells, titer $\log_{10}$6.9 $CCID_{50}$/ml
2. Mumps virus (Mano strain) 5th passage in Vero cells, titer $\log_{10}$7.4 $CCID_{50}$/ml
3. Mumps virus (Sano strain) 4th passage in Vero cells, titer $\log_{10}$7.2 $CCID_{50}$/ml

Each virus was passed only once on Vero cells. The titers of our stock viruses were $\log_{10}$7.0. $\log_{10}$7.2 and $\log_{10}$7.4 $CCID_{50}$/ml, respectively

Cell cultures

Primary GMKC were prepared from a frozen stock of cells. The cells were fully investigated for the absence of adventitious agents by passage of cell lysates in BSC-1, FL, HDC, RK13 and primary guinea-pig kidney cells. The growth medium consisted of Earle's medium containing 0.5% lactalbumin hydrolysate, 8% inactivated serum, 100 μ/ml streptomycin and 100 μg/ml of kanamycin. Maintenance of cell culture for virus growth was performed using Eagle's BME (Earle' base) with 2% inactivated calf serum and the antibiotics as reported before. For attenuation of the virus, HDC(MRC-5) at doubling passages 14 to 24 were cultivated in Eagles's MEM containing 4% inactivated baby calf serum. When cpe was observed, the culture medium was replaced by the same medium from which calf serum was omitted. For titration of virus, Vero cells, a stable cell line of GMKC, was propagated according to the method described by Sasaki *et al.*[1]

Plaquing

Plaquing in Vero cells of wild and attenuated mumps S-12 strain was performed under fluid tragacanth gum overlay as we have previously reported.[2]

Titration of virus

Titration was carried out using serial 10-fold dilutions of virus in Vero cells. Four tubes of cells were used per dilution. inoculum was 0.1 ml of virus diluted in growth medium without serum. The cultures were incubated at 35°C when wild viruses were assayed and at 32°C in the case of attenuated viruses. The final reading was made on the 10th day. The end-point titration by hemadsorption with guinea-pig red blood cells was also performed the same day.

Hamsters

Multiparous pregnant Syrian hamsters were obtained from our local colony. Newborn (<24 h old) hamsters were given intraperitoneal (i.p.)inoculations of 0.1 ml of different stock viruses. Litters were selected randomly for each virus. Animals were anaesthetized and sacrificed at days indicated in Table 5. They were decapitated and blood was collected in heparinized tubes. Brains were removed aseptically, pooled, homogenized and a 50% suspension in growth medium was stored frozen at-70°C. Before assay, the samples were thawed and clarified by centrifugation at 500 g for 15 min. The clarified suspension (0.1ml) was inoculated into four tubes of monolayers of Vero cells for the isolation of the free virus. The monolayers were incubated at 35°C and were observed for cpe for 2 weeks. The presence of cell-associated virus was not studied in this investigation.

Monkeys

Cynomolgus monkeys of 2 to 3 kg in weight were healthy primates imported from the Far East. Monkeys free of mumps HI antibody were selected for this study.

Mumps antisera

Two hyperimmune antisera against wild Mano strain and Hoshino-L32 vaccine strain were produced in rabbits. Human anti-mumps serum was received from Dr Parkman (NIH) U.S.A. Convalescent serum of the patient from whom the mumps virus was isolated and was obtained 1 month after recovery from the infection.

Serology

Hemagglutination inhibition (HI) test was applied by using the sensitive HI test developed by Albrecht & Kitch.[3] The neutralization test (NT) was performed in Vero cell cultures, and 100-200 $CCID_{50}$ of challenge virus as test dose were mixed with dilutions of sera. The reading of antiboldy titers was done on the 10th day following incubation at 35°C. The titer was noted as the highest initial dilution of serum which inhibited the appearance of cpe. Sera were treated by kaolin and guinea-pig red blood cells (GPRBC) for removing inhibitors and nonspecific agglutinins respectively. In the HI test, GPRBC was used an indicator for the test.

Preparation of attenuated S-12 mumps vaccine

Using the 14th subculture growth of S-12 strain in HDC as a working seed, a batch of mumps vaccine was prepared. The seed virus was mixed with a suspension of HDC at a doubling passage of 18 to 50 Roux bottles before plating. The cultures were incubated at 35°C for 48 h until the monolayer cell was completed, they were then transferred to 32°C. The growth medium was MEM (Earle' base) containing 4% calf serum. After 6 days, when the cpe was observed in most parts of the cell surface, maintenance medium was completely removed and was replaced by a serum-free medium of the same composition. Next day, the supernatant fluid was removed and centrifuged at 3000 rpm for 30 min in order to eliminate the cell debris. samples were taken for titration and other controls. The suspension of virus was blended with a suitable stabilizer and the product was the lyophylized. The vaccine was subjected to a bacteriological test for sterility and small animal inoculation for assuring the safety of the product. The vaccination dose was 0.5 ml containing a $\log_{10}$ 5.0 $CCID_{50}$/dose.

Vaccinees and vaccination

Twenty seronegative male volunteers of over 20 years of age were inoculated with one dose of vaccine subcutaneously.

Thirty children of good health, aged 1 to 5 years, with no previous history of mumps infection were also inoculated with 0.5 ml of the vaccine subcutaneously. To study the antibody response, capillary blood samples were taken immediately before immunization and 5-6 weeks later.

Results

Biological characteristics of mumps virus, attenuated S-12 strain

1. Adaptation to CMKC and HDC. As shown in Table 1, the virus which was isolated directly in GMKC, yielded a relatively high titer after three passages in this cell culture. Incubation time was 5 days at 35°C. The virus was then passaged in HDC(MRC-5). After six successive passages in HDC, with an incubation period of 5-7 days at 35°C, the virus reached the titer of 5.0 $\log_{10}$/ml. However, the virus yields remained stationary between the 6th and 10th passages in HDC. The increase of titer by one log or more was obtained when the passage of virus was made by end-piont dilution, between the 11th to 14th passage.

Table 1. History of adaptation and attenuation of the S-12 strain of mumps virus in cell culture

Type of cell	*Passage level*	*Temperature of incubation (°C)*	*Incubation period (days)*	*Virus titer $CCID_{50}$ $\log_{10}$/ml*
	1	*35*	*6*	*ND*
GMKC	*2*		*5*	*3.75*
	3		*5*	*4.60*
	1		*6*	*4.50*
	2		*5*	*4.50*
	3		*7*	*3.75*
	4		*7*	*4.12*
	5		*6*	*4.87*
HDC(MRC-5)	*6*		*5*	*5.20*
	7		*4*	*4.75*
	8		*5*	*5.12*
	9		*5*	*5.20*
	10		*5*	*5.30*
	11	*32*	*5*	*6.62*
	12		*6*	*6.50*
	13		*5*	*6.0*
	14		*5*	*6.25*

ND, not done.

Table 2. Identification of the isolated virus

	Immune sera			
Antigens	*Wild mano strain (RABBIT)*	*Hoshino strain L-32 (rabbit)*	*Mumps antiserum Dr Parkman (human)*	*Convalescent serum Patient S-12*
Wild mumps Sano strain	*16X*	*32X*	*128X*	*512X*
Isolated virus S-12 strain GMK/3,MRC-5/4	*64X*	*64X*	*512X*	*2048X*

2. *Identification of virus.* The results of comparative seroneutralization tests of the isolated virus and the reference Sano strain by using three mumps antisera and convalescent serum of the patient are reported in Table 2 which indicated that the isolated organism is a mumps virus. The strain has been called wild mumps, S-12 strain.

3. *Marker test.* The variation between size of plaques in Vero cell monolayers, of wild and attenuated strains of S-12 mumps virus is illustrated in Fig. 1. while the wild strain yielded plaques of 2-3 mm diameter, the attenuated S-12 strain produced plaques of about 1 mm diameter. This finding is similar to those reported by Flanagan *et al.*[4] in the case of the Jeryl Lynn strain, Yamanishi *et al*[5]. for Urabe-9 strain and by Glück *et al*[6]. for the Rubini strain.

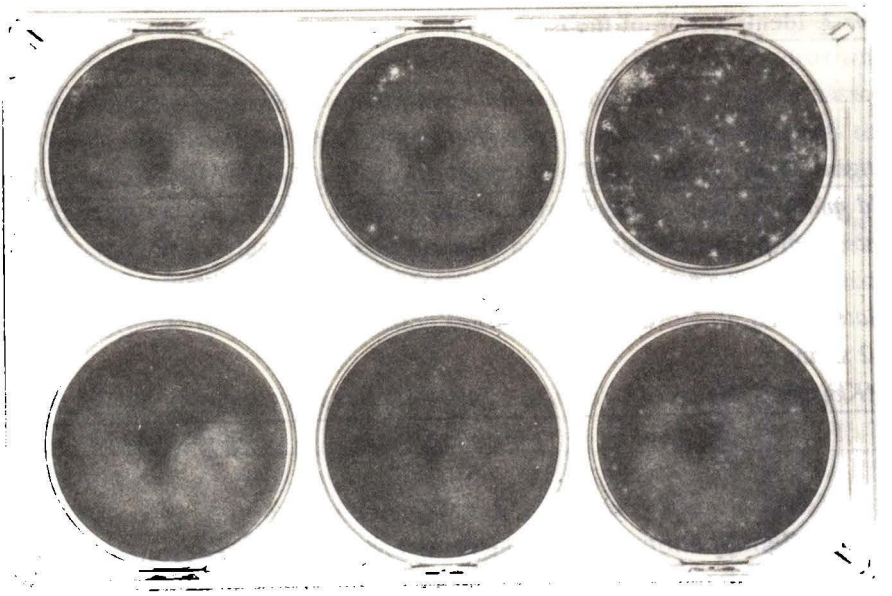

Figure 1. *Upper row: plaques of wild mumps virus, Sassani strain in Vero cells culture after 6 days at 35°C. plaque size 2-3 mm. Lower row: plaques of attenuated mumps virus, Sassani strain in Vero cell culture after 6 days at 35°C. Average plaque size 1 mm.*

Experimental infection of monkeys

Two wild S-12 and Sasazaki strains and two attenuated S-12 and Hoshino-L32 strains were employed in this study. The titer of these four viruses was adjusted to approximately $10^{5.0}$ $CCID_{50}$/ml. From each virus suspension two monkeys received 1 ml in five spots on the left parotid (0.2 ml at each spot), two monkeys were injected intracerebrally with 0.5 ml and finally one monkey was inoculated with 0.25 ml in cisterna cerebellomedularies. The cohabiting non-inoculated controls were kept with monkeys injected intramuscularly or on parotid. The period of observation was 21 fdays. During this period of time the monkeys did not show any clinical symptoms of paralysis, but did show transient swelling of parotid glands, as indicated below. The monkeys were killed under anesthesia and the results are summarized in Tables 3 and 4. slight unilateral

swelling of the parotid glands were observed in three out of four monkeys injected with S-12 wild strain and in one out of four monkeys inoculated with Sasazaki wild strain, intramuscularly or in parotid. Manifestation of central nervous disorders have not been clinically revealed in any monkeys regardless of type or route of injection of virus. Monkey No. 29 died while under anesthesia shortly after intracerebral inoculation of Sasazaki strain. Regarding the histo-Pathological survey of the central nervous system (CNS) and parotid glands, monkeys inoculated intracerebrally with either attenuated or wild strain did not show any pathogenic effects in CNS or the parotid glands. on the contrary, both monkeys inoculated intramuscularly or in the parotid gland with wild Sasazaki strain, and both the monkeys injected in the parotid gland and the monkey inoculated intramuscularly with wild S-12 strain showed moderate to minimal lesions with engorgement of the capillaries and minimal perivascular cuffings. Of the cohabiting noninoculated controls, monkeys 32 and 34 kept in cages with monkeys inoculated with wild S-12 strain intramuscularly or on the parotid-showed a significant rise of serum HI antibody titer. Serum HI antibody of inoculated monkeys, before injection and 21 days later, are presented in Tables 3 and 4.

Inoculation of day-old hamsters

Both wild and attenuated S-12 strains, Hoshino-L32 vaccine strain and Sasazaki wild strain were injected intracerebrally to day-old hamsters. Each virus suspension was inoculated to four litters of baby hamsters. The results, which are summarized in Table 5, indicate that the hamsters did not show any clinical symptoms during the period of observation. The brains of hamsters injected with wild Sasazaki or S-12 strains and sacrificed at the date indicated in Table 5 did not show any histopathological changes, although mumps virus was isolated 12 days after inoculation of the wild Sasazaki strain. For both attenuated S-12 and Hoshino-L32 strains, the brain of one hamster showed histopathological changes at 12 and 16 days post-inoculation respectively. Capillaries were engorged with blood cells, in some areas mononuclear cells were infiltrated in perivascular spaces. inflammatory cells infiltrated focal areas surrounding the leptomeninges. Mumps virus was isolated from the brain suspension 4 days after i.p. inoculation of Hoshino-L32 strain. The HI antibody titer of serum of hamsters 7 to 8 weeks following i.p. inoculation of wild or attenuated mumps strains were 5 $\log_2$-6 $\log_2$.

Table 3. Comparison of clinical reactions, histopathological changes and antibody responses of monkeys inoculated with two wild strains of mumps virus

virus	Monkey No.	Inoculation route	Clinical reactions: Swelling of Parotid glands	Clinical reactions: Nervous system disorders	Histopathological finding: Parotid glands	Histopathological finding: Central nervous	Antibody response: HI titer log_2: Pre-inoc. (0 day)	Antibody response: HI titer log_2: Post-inoc. (21 days)
Wild S-12	18	i.m.	–	–	–	–	<2	4
CMKC-3/	19		*	–	+	–	<2	3
HDC-2	20	I.parot.	*	–	+	–	<2	5
	21		*	–	+	–	<2	5
	22	i.cereb.	–	–	–	–	<2	5
	23		*	–	–	–	<2	6
	24	i.cistern. cereb	–	–	–	–	<2	4
Sasazaki	25	i.m.	–	–	+	–	<2	5
strain	26		–	–	+	–	<2	5
Vero/4	27	i.parot.	*	–	+	–	<2	6
	28		–	–	+	–	<2	4
	29	i.cereb.	+				<2	–
	30		–	–	–	–	<2	4
	31	i. cistern. cereb.	–	–	–	–	<2	3
Controls	32		–	–	–	–	<2	3
	33	–	–	–	–	–	<2	<2
	34		–	–	–	–	<2	4

* Slight and transient swelling of parotid glands of one side.
+ Monkeys died following inoculation under anaesthesia.
+ Moderate to minimal lesions with engorgement of the capillaries and minimal perivascular cuffings.

Table 4. Comparison of clinical reactions, histopathological changes and antibody responses of monkeys inoculated with two attenuated strains of mumps virus

virus	Monkey No.	Inoculation route	Clinical reactions		Histopathological finding		Antibody response: HI titer $\log_2$	
			Swelling of Parotid glands	Nervous system disorders	Parotid glands	Central nervous	Pre-inoc. (0 day)	Post-inoc. (21 days)
Attenuated	1	i.m.	–	–	–	–	<2	3
S-12 strain	2		–	–	–	–	<2	3
(GMK-3/	3	i. parct.	–	–	–	–	<2	5
HDC-14)	4		–	–	–	–	<2	5
	5	i. cereb.	–	–	–	–	<2	3
	7	i. cistern. cereb.	–	–	–	–	<2	6
Hoshino	8	i. m.	–	–	–	–	<2	3
L-32 strain	9		–	–	–	–	<2	4
	10	i. parot.	–	–	–	–	<2	5
	11		–	–	–	–	<2	4
	12	i. cereb.	–	–	–	–	<2	3
	13		–	–	–	–	<2	4
	14	i. cistern. cereb.	–	–	–	–	<2	6
Controls	15		–	–	–	–	<2	<2
	16	–	–	–	–	–	<2	<2
	17		–	–	–	–	<2	<2

Table 5. Histological findings in one central nervous system of day-old hamsters injected intraperitonealy with virulent or attenuated strains of mumps virus.

Hamster no.	Virus strain	Virus titer $CCID_{50}$ log_{10}/ml	Inoculum per hamster	Clinical symptoms	Sacrificed onday... post inoculation	Histo-pathology (brain)	Virus isolation (brain)	Antibody response HI titer log_2
1	Wild				12	–	+	ND
2	Sasazaki	6.0	0.1 ml		16	–	ND	ND
3	Vero/4			–	21	–	ND	ND
4					52	–	–	6.0
5					7	–	–	ND
6	S-12GMK/3	6.0	0.1 ml		12	–	–	ND
7	MRC-5/2			–	16	–	–	ND
8					47	–	–	5.0
9	Attenuated				4	ND	+	ND
10	Hoshino	5.0	0.1 ml	–	16	*	ND	ND
11	L-32				44	–	–	6.0
12					50	–	–	ND
13					7	–	–	ND
14	S-12GMK/3	5.0	0.1 ml	–	12	*	–	ND
15	HDC/14				16	–	–	ND
16					47	–	–	5.0

* Capillaries were engorged with blood cells in scattered areas, lymphocytes, predominantly mononuclear cells, were infiltrated in prevascular spaces. Inflammatory cells were infiltrated in focal areas in surrounding leptomeninges.
ND, not done.
+ Virus was isolated in Vero cells.

Quality control of the vaccine

The potency and safety of the vaccine was evaluated according to the WHO Minimum Requirements of live attenuated mumps vaccine[7] and subjected to bacteriological, small laboratory animal inoculations and monkey infectivity tests. The safety of vaccine was confirmed by all the above mentioned tests.

Response to attenuated mumps S-12 strain

The inoculation of one dose of lyophilized mumps vaccine containing 100000 ID_{50} of the virus in volunteers did not induce fever, local or systemic reactions. In 30 susceptible children inoculated with one dose of MMR prophylactic containing 20000 ID_{50} of AIK strain measles virus, 100000 ID_{20} of S-12 mumps attenuated virus strain and 2000 ID_{50} of Takahashi strain of rubella virus all developed in HDC(MRC-5), stabilized with a proper stabilizer and lyophilized. Local or systemic reactions such as swelling of parotid glands were not reported. Capillary blood samples taken at 4-5 weeks after vaccination showed seroconversion of 93% for the mumps component of MMR. The mean neutralizing antibody titre for mumps were 1/32-1/64.

Discussion

The attenuation of vaccine strains of mumps virus during the last four decades has been accompanied mainly by the passage of virus in a chick embryo cavity. Enders *et al*[8]. reported that after a single egg passage the virus does not cause clinical reactions. Buynak *et al*[9]. observed that attenuation of mumps virus takes place after 17 passages in CEF, but, as reported by Hosai *et al*.,[10] attenuation of a virus was complete after 12 passages in chorioallantoic cavity and for Yamanishi *et al*[11]. the attenuation occurred after only four passages in the chick embryo cavity. Sasazaki *et al*.[12] were able to isolate a cold variant of the mumps virus, called Hoshino-L32 strain, after four passages of cloned virus in CEF at 32°C. Leningrad-3(L-3) strain, isolated and attenuated in guinea-pig kidney cell culture and Japanese quail embryo cell cultures,[13] was finally adapted to CEF. The third passage material was therefore established as the master seed for the manufacture of vaccines.[14] It is therefore evident that most of the vaccine strains of mumps already in use have been originally isolated from infected chick embryos or allantoic fluids of SPF embryonated ben's eggs, and the vaccines are

produced in CEF. These vaccines, whilst being fully safe and immunogenic, may sensitize children who are sensitive to vaccines. In addition, their production requires specific pathogen-free eggs[7] containing traces of chick proteins, attempts to produce mumps vaccine in human cells have been reported by some investigators. Ikic *et al.*[15] adapted a mumps virus, isolated by three serial passages in amnion cavity of 8-day-old embryonated eggs, in WI-38 human diploid cells, and Gluck *et al.*[6] attenuated the Rubini wild mumps strain by 15 serial passages at 35°C in HDC(MRC-5). This strain was shown to be immunogenic and safe in susceptible children.

In the present study, the virus was isolated directly in GMKC, followed by serial passages in HDC. As shown in Table 2, the titer of virus after 10 passages in HDC increased by about one $\log_{10}$; at this stage cloning of virus by end-point dilution at 32°C was performed. it is worth mentioning that all passages were done by adding inoculum of the virus to the suspension of HDC before plating cells. It was observed that by inoculating the virus in monolayers of HDC, the titer would often be lower by one $\log_{10}$ or more.

The mumps attenuated S-12 strain can be distinguished from its wild strain homologus by plaque size. Thus, after 6 days incubation at 35°C under a fluid overlay, the S-12 wild strain yielded plaques of 2 to 3 mm, while the attenuated S-12 strain produced plaques of about 1 mm in diameter.

The neuropathogenesis of virus, inoculated intraperitoneally in day-old hamsters was also studied. The wild S-12 strain, as well as the wild Sasazaki strain, did not reveal any pathological lesion in brains 7 to 52 days after inoculation, however, mumps virus was isolated from the pool of brains of one litter of baby hamsters 12 days after inoculation with the wild Sasazaki strain. Both mumps attenuated strains, S-12 and Hoshino-L32, provoked histopathological changes 12 to 16 days after inoculation in one out of four hamsters. The free virus was isolated from the brain of one animal injected with Hoshino-L32 but it was not titrated.

persistence of the mumps virus in the brains of newborn mice 100 days after the initial infection has been reported by Hayashi *et al.*[16] When the mumps wild S-12 or virulent Sasazaki strains were inoculated into monkeys by the intramuscular, intraperitoneal or intracerebral routes, the transient and slight swelling of the parotid glands were mainly moderate to minimal lesions with engorgement of the capillaries and minimal perivascular cuffings. No change was recognized in brain tissue of any of the monkeys, regardless of the route of inoculation of virus. in all monkeys a significant rise of HI antibody titer was

recorded. The rise of antibody was also noticed in two monkeys cohabiting with primates inoculated with wild virus.

The clinical and histopathological findings in monkeys inoculated with either attenuated S-12 or Hoshino-L32 strains by intramuscular, intraparotid, intracerebral or intracistrnal route were quite different from those of animals inoculated with wild type viruses. Swelling of parotid glands and histopathological changes were not observed. HI antibody titer was increased in serum of all monkeys, except the controls cohabiting with inoculated primates. The monkey test as well as the characteristic marker of mumps virus and the reduction of plaque size following successive passages in HDC all indicate that the S-12 strain is more attenuated than its parent S-12.

A chief point of concern in this study is the higher virulence of the candidate vaccine strain for baby hamsters than its original wild strain. Similar finding has been reported by Kilham & Margolis[17] who noticed that Jeryl Lynn, as it is employed in a live attenuated vaccine (Mumpsvax), appeared more virulent for baby hamsters than the natural strain.

A possible explanation for this finding suggested by these authors is that the vaccine strains were attenuated in respect of adult hosts only. Both Jeryl Lynn and Hoshino-L32 strains are attenuated in embryonated hen's eggs as well as in chick embryo fibroblasts. It is worth mentioning that according to Wolinsky *et al.*,[18] neuroadapled mumps virus produces systemic infection in newborn hamsters after i.p. inoculation. The development of neutralizing and HI antibodies in serum correlates with the clearance of virus from most systemic sites. However, the authors have found persistence of the virus in both brains and kidneys late in this infection. is this finding applicable to the mumps vaccine strains which have lost their reactogenicity and pathogenicity for primates and for man? whether or not we can parallel the pathogenesis of mumps infection in human and in newborn hamsters remains to be investigated more thoroughly.

It is interesting at this point to note that several cases of mumps meningitis following mumps vaccination have recently been reported in Canada, japan and Europe, where Urabe or Jeryl Lynn strains have been incorporated in trivalen measles-mumps-rubella vaccines.[19-24] These two strains are widely used in the immunization of children in various parts of the world. The Hoshino-L32 strain which has been used in over 700000 doses in Japan has so far been safe and no cases of post-vaccination meningitis have occurred.[19] The same strain used in Iran since 1988 for the immunization of more than 100000 children aged 1 to 5 years in MMR prophylactic or as a monovalent mumps vaccine has proved to

be safe and immunogenic.[25]

In a limited field trial, this new mumps vaccine strain has been shown to be safe, no reactogenic and quite immunogenic for adults and children.

At this stage, based on our observation in nonkeys, in laboratory animals and in man-while we confirm the safety of the S-12 strain of mumps virus attenuated in HDC-we would like to repeat the suggestion of Dr J. Steigman[26] who said: 'A immunizing agent need only be shown to be safer than exposure to the disease in nature'.

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