**SUPPLEMENT TO THE PATHOGENICITY AND IMMUNOGENICITY
OF AN ATTENUATED VACCINIA VIRUS, STRAIN
DIs, IN CYNOMOLGUS MONKEYS**

ISAMU TAGAYA, HIROKO AMANO, TOSHIHIKO KOMATSU,
NOBUYUKI UCHIDA* AND HIDEO KODAMA*

*Department of Enteroviruses and *Department of Pathology, National Institute
of Health, Nakato, Musashimurayama, Tokyo 190-12, Japan*

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SUMMARY: The pathogenicity of the DIs virus was compared with those of three other vaccinia virus strains, Lister (Elstree), Ikeda and IHD, by intracerebral inoculation into cynomolgus monkeys. By inoculation of a large amount of the virus into the thalamus and cisterna, the animals receiving the DIs virus remained normal, whereas those receiving the other viruses showed severe clinical symptoms such as anorexia, apathy, coma, and paralysis, and were found moribund or dead within a few days. Histological studies also revealed a big difference in the severity of lesions induced by the DIs virus and those by the other three vaccinia viruses, although the main common finding was leptomeningitis purulenta acuta.

Immunization of cynomolgus monkeys with a single shot of the DIs viurs via the sc, id or im route, followed by a percutaneous challenge with the Ikeda virus, revealed that the skin resistance had not been established (Tagaya et al., 1973). Studies on the dynamics of circulating hemagglutination-inhibiting and neutralizing antibodies, however, indicated that the immunized animals responded more readily and strongly to the challenge with the Ikeda virus than did non-immune animals. This may suggest that the DIs virus induced basic immunity in monkeys, although circulating neutralizing antibody was not detectable and skin reactions by the Ikeda virus were only slightly modified. Thermolabile vaccinia virus-neutralizing activity in the serum also appeared or increased by inoculation with the DIs virus as well as with the Ikeda virus. The immunological role of this substance awaits further studies.

INTRODUCTION

Preliminary studies indicated that an attenuated mutant, DIs, of the Dairen-I (DI) strain of vaccinia virus induced mild skin lesions in cynomolgus monkeys by scarification of the skin or intradermal inoculation. Hemagglutination-inhibiting (HI) and neutralizing antibodies were shown in the sera obtained from 1 to 6 weeks post-inoculation (p.i.) (Kitamura, Kitamura and Tagaya, 1967). In these studies, however, multi-spot inoculations were given to each monkey, and it was considered necessary to examine in more detail the pathogenicity and immunogenicity of the virus in mon-

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多ヶ谷 勇・天野 浩子・小松 俊彦 (国立予防衛生研究所 腸内ウイルス部)
内田 信之・児玉 栄夫 (国立予防衛生研究所 病理部)

keys before a possible applicability of the mutant to human vaccination is considered. Further studies were carried out to examine the pathogenicity of the DIs virus in monkeys, inoculating a large amount of the virus intracerebrally, intravenously or percutaneously. It was revealed that the virus was also highly attenuated to cynomolgus monkeys. Immunization of monkeys with a single shot of the DIs virus by various routes, followed by percutaneous challenge with the ordinary calf lymph smallpox vaccine, was then carried out. It was indicated that the DIs virus could not immunize monkeys strong enough to resist percutaneous challenge with the ordinary vaccinia virus, although circulating antibody response suggested the induction of basic immunity by the DIs virus. A summary of the studies was reported at the International Symposium on Smallpox Vaccine, Bilthoven 1972 (Tagaya *et al.*, 1973). The present report deals with supplementary data on the pathogenicity of the DIs virus in comparison with the other three strains of vaccinia virus as well as complete data on the dynamics of circulating antibodies in monkeys by immunization with the DIs virus and challenge with the Ikeda virus, which were not available at the time of the symposium.

MATERIALS AND METHODS

Viruses: The DI and DIs viruses at the 24 to 26th passage through the chorio-allantoic membrane (CAM) of conventional fertile hen's eggs were used. The Ikeda, Lister (Elstree) and IHD (hemagglutinin positive) strains of vaccinia virus were used as the homogenate of infected CAM's of the 2nd, 3rd and first passage, respectively, performed in our laboratory from the following original seeds. The original Lister virus was the lyophilized International Reference virus; the original Ikeda virus a lot of lyophilized calf lymph vaccine prepared by the Kitasato Institute. The IHD virus kept in our laboratory was with the passage history M25E3 (25 passages through the mouse and 3 passages through CAM). A commercial lot of liquid calf lymph vaccine prepared with the Ikeda virus was also used for challenging DIs-immunized monkeys.

Titration of infectivity: The plaque titration of the virus in CE cell monolayers was described elsewhere (Ueda *et al.*, 1972; Tagaya *et al.*, 1974). For the recovery and quantification of virus from inoculated monkeys the serum or a 10% homogenate of each organ was diluted decimally and 0.2 ml each was inoculated onto 3 to 4 CAM's of 12-day egg per dilution. Phosphate-buffered saline (PBS), pH 7.4, containing gelatin at 0.2% was used as the diluent for virus materials.

Titration of circulating neutralizing antibody: This was described earlier (Ueda *et al.*, 1972).

Titration of HI antibody: The micro-method in the disposable tray was used with PBS as diluent. For hemagglutinin (HA) titration, duplicate serial twofold dilutions of the antigen (homogenate of DI-infected CAM's) were made in 0.025 ml with a standardized Takátsy loop, starting from a 1:10 dilution. To one series, 0.025 ml each of 1:30 diluted normal rabbit serum and to the other 0.025 ml each of 1:30 diluted antivaccinia rabbit serum was added. The sera were inactivated at 56 C 30 min before use. To each well 0.025 ml of a 0.2% erythrocyte suspension from vaccinia HA-sensitive fowls was then added and the plate was shaken on a vibrator for 5 sec. After 60-min incubation at 37 C the results were read and the highest dilution of the antigen showing complete hemagglutination pattern in the normal rabbit

serum was taken as the endpoint. The specificity of this hemagglutination was checked by its inhibition in the immune rabbit serum line. For HI test serial twofold dilutions of a serum was set up in duplicate. To one series 0.025 ml of 4 units of HA was added and to the other an equal amount of normal rabbit serum diluted 1:30 was added, which served as test serum control to examine the presence of non-specific hemagglutination. The plate, after being shaken, was incubated at 36 C for 30 min and then 0.025 ml of a 0.2% fowl erythrocyte suspension was added to each well. The plate was shaken and incubated at 36 C for 60 min and HI titer was expressed as the highest dilution of a serum showing complete inhibition of hemagglutination. When serum control showed non-specific hemagglutination, 0.5 ml of the original serum was absorbed with 0.025 ml of packed fowl erythrocytes at 37 C for 30 min. The red blood cells were removed by centrifugation at 2,500 rpm for 7 min and the supernatant served as absorbed serum. Parallel titration of HA used for the test was always set up.

Inoculation of monkeys: Cynomolgus monkeys of both sexes with body weights of about 2 to 3 kg were used. Intracerebral inoculation was carried out by injecting 0.25 ml of a test material into the cisterna and 0.5 ml into one side of the thalamus. Monkeys were sacrificed at moribund or 10 days p.i. Bleeding was made before inoculation and at autopsy. Histological examinations were made by the ordinary technique with the organs obtained at autopsy.

Fluorocarbon treatment of vaccinia-infected CAM homogenate: Daiflon S3 (trifluorotrichloro-ethane, Daikin Kogyo Co. Ltd.), cooled in a deep freezer at -20 C was added to homogenate of infected CAM's at a final concentration of 50% and mixed in a blender under cooling with ice. The water phase was separated by light centrifugation. Pre-cooling of Daiflon S3 was necessary to minimize the loss of the infectivity of the homogenate of DIs-infected CAM's.

RESULTS

Intracerebral Inoculation of Cynomolgus Monkeys with the DIs Virus or Three Other Strains of Vaccinia Virus

As reported in a previous publication (Tagaya et al., 1973), cynomolgus monkeys inoculated with the DIs virus ($10^{8.0}$ TCID₅₀/ml) intravenously or percutaneously remained normal except for local reddening 6 to 8 days p.i. in those inoculated by multiple pressures; but those inoculated intracerebrally (intrathalamic 0.4 ml and intracisterna 0.25 ml) showed fever 3 to 6 days p.i. with lassitude and anorexia, from which they appeared to recover later. An experiment was designed then to compare the pathogenicity of the DIs virus with those of the other three vaccinia viruses by intracerebral inoculation into cynomolgus monkeys. Homogenate of infected CAM's was treated two or three times with fluorocarbon before use. The results are shown in Table I, which includes those on the DIs virus reported in a previous publication (Tagaya et al., 1973). The animals inoculated with the Ikeda, Lister or IHD viruses showed severe clinical symptoms from 1 to 2 days p.i. Out of three monkeys inoculated with the IHD virus, one was found dead the day following inoculation and excluded from evaluation, because the death was considered to have been due to inoculation trauma. The other two monkeys, however, were seriously ill; one of them was found dead and the other was moribund 2 days p.i. Those inoculated with the

TABLE I
Inoculation of cynomolgus monkeys with four strains of vaccinia virus

Virus strain and dose*	Monkey		Days p.i. dead or sacrificed	Virus recovery** from				HI antibody titer	
	Number	Sex	Body wt (kg)	Serum	Brain	Cervical cord	Lumbar cord	before inoculation	at autopsy
Ikeda 107.3	9708	f	2.14	— [#]	5.9	5.5	>6.0	<2	<2
	9709	f	2.00	—	7.5	5.6	>6.0	<2	<2
	9712	f	2.50	—	5.6	5.5	>6.0	<2	<2
Lister 107.4	9718	f	2.52	—	4.7	5.3	1.2	<2	<2
	9792	f	2.94	—	5.9	5.8	4.8	<2	<2
	9796	m	2.64	ND	6.0	5.4	4.3	<2	ND
			3+ ^{##}						
IHD 107.4	9713	f	2.52	—	4.7	>6.0	5.6	<2	<2
	9714	m	1.50	ND	3.8	>6.0	5.4	<2	ND
	9717	m	1.64	ND	ND	ND	ND	<2	ND
DIs 107.3	9698	f	2.80	—	—	—	—	<2	16
	9699	f	3.24	—	—	—	—	<2	32
	9707	f	2.08	—	—	—	—	<2	16

* Inoculum was the homogenate of infected CAM's after fluorocarbon (Daiflon S3) treatment with the infectivity indicated in terms of pock forming units (po.f.u.)/ml. Each monkey received 0.5 ml intrathalamically and 0.25 ml intracisternally.

** Expressed as po.f.u./ml.

— means negative by inoculating undiluted materials of a 10 % homogenate of brain (portion of the precentral gyrus), cervical cord and lumbar cord, or serum. Some of the specimens were also assayed in CE monolayers to confirm the absence of plaque forming virus.

+ means the monkey was found dead on the day p.i. indicated. The monkey No. 9717 was considered to have died by inoculation trauma.

Ikeda or Lister viruses were also moribund or deceased 3 to 4 days p.i., whereas those inoculated with the DIs virus remained apparently normal without rise of body temperature during the observation period of 10 days. Vaccinia virus was isolated from all specimens of the brain and spinal cord of the animals inoculated with the Ikeda, Lister or IHD viruses; the virus contents of these organs were high except the lumbar cord of one monkey inoculated with the Lister virus. Vaccinia virus was not detectable in the serum of these animals. Attempts at virus isolation with the specimens collected from those inoculated with the DIs virus 10 days p.i. were unsuccessful. HI antibody was not detectable in the serum of those inoculated with the Ikeda, Lister or IHD viruses due to the early death or sacrifice of the animals. Rise in circulating HI antibody was confirmed in the serum of those inoculated with the DIs virus, indicating multiplication of the virus in the animals in spite of no development of clinical symptoms.

The results indicate that the DIs virus is far less pathogenic to cynomolgus monkeys by intracerebral inoculation than the other three vaccinia strains, two of which have been extensively used for human vaccination in this country or in many other countries in the world.

*Histological Examination of Organs of Monkeys Inoculated
with the DIs or Other Vaccinia Virus Strains*

There was no other particular gross findings than turbidity of the leptomeninges and dilatation of the meningeal vessels in the central nervous system. Major parts examined microscopically were parietal lobe including sulcus centralis, basal ganglia, midbrain, cerebellum with pons, medulla, cervical and lumbar enlargements of the spinal cord in the central nervous system, and a few visceral organs such as liver, spleen, lung, lymph nodes, etc. The main pathological change was leptomeningitis in all the monkeys examined. The animals inoculated with the Ikeda, Lister or IHD viruses, however, had marked leptomeningitis of similar severities. These changes were far severer than those in the animals inoculated with the DIs virus. Microscopic changes in the leptomeninges of the animals inoculated with the virulent viruses were typical fibrino-purulent leptomeningitis with marked congestion in the vessels of the subarachnoid spaces and with fibrino-purulent exudate containing numerous polymorphonuclear leukocytes, moderate lymphocytes and mononuclear cells. The exudate was abundant in the sulci of the severe cases such as Nos. 9712, 9714, and 9796 (Fig. 1). Purulent inflammation extended to the choroidal plexus (Nos. 9712, 9713, and 9796) (Fig. 2) and also continued slightly under the subependymal space (No. 9712).

As anticipated from the clinical observations, the monkeys inoculated with the DIs virus revealed only slight leptomeningitis with infiltration with predominant round cells around the vessels in the pia-arachnoid spaces (Fig. 3). There were no or a few polymorphonuclear leukocytes in such infiltration. The difference in the type of the infiltrating cells in the leptomeninges between the DIs virus group and three other virus groups of animals may have been due to the different pathogenicities of the viruses and in part to the time of autopsy after inoculation. There were hemorrhage and acute purulent inflammation in the direct vicinity of the inoculation site in the IHD, Lister and Ikeda virus groups of animals and slight ones in the DIs virus group. Besides these findings there observed in all the monkeys extremely slight edema around the small vessels, but neither changes in the neurons nor demyelination in the paren-

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Acute purulent leptomenigitis with non-specific panangitis of small artery in the brain of a cynomolgus monkey (No. 9796) inoculated with the Lister virus.

Fig. 2. Acute purulent leptomenigitis extending to choroid plexus in the same monkey shown in Fig. 1.

Fig. 3. Slight subacute leptomenigitis in the brain of a cynomolgus monkeys (No. 9698) inoculated with the DI1 virus.

Fig. 4. Small accumulation of polymorphonuclears like abscess in the lymph follicle of the spleen of a cynomolgus monkey (No. 9713) inoculated with the IHD virus.

chyma of the brain except the inoculation site.

The spleen of the animals inoculated with any of the three viruses other than the DIs virus revealed histologically acute splenitis. Although there was a little difference in the severity of the changes among monkeys, they showed moderate congestion, accumulation of remarkably a large number of polymorphonuclear leukocytes in the cellular red pulp and slight atrophy of lymphoid tissues with decreased number of the reaction centers. Interesting findings were that the reaction centers were sometimes necrotic with a small accumulation of polymorphonuclear leukocytes showing an appearance of small abscess (Fig. 4). On the contrary the spleens of the animals inoculated with the DIs virus did not show any changes such as acute infectious splenitis.

The lungs of the two monkeys each inoculated with the IHD or Lister viruses respectively showed slight inflammatory changes in the alveolar septa.

*Antibody Response of Cynomolgus Monkeys after Immunization
with the DIs Virus and Challenge with the Ikeda Virus*

Twelve monkeys were divided into four groups of three monkeys; nine of them were inoculated with homogenate of DIs-infected CAM's treated twice with fluorocarbon before use. The infectivity of the virus preparation was $10^{7.7}$ PFU/ml. The first group received 1.0 ml subcutaneously into the right upper arm, the second group 0.2 ml intradermally into the right upper arm and the third group 1.0 ml intramuscularly into the right shoulder. The 4th group were left uninoculated and kept in the same room as room mates. Each monkey was kept in a separate cage. Those inoculated subcutaneously or intramuscularly showed no recognizable local reaction, while each of those inoculated intradermally showed a pinpoint induration of about 2 mm diameter, followed by scab formation. All the monkeys except one remained in good conditions until the challenge with the Ikeda virus 13 weeks later. One animal in the first group died 7 weeks p.i. due to an unspecified cause. After 13 weeks these monkeys were inoculated with a commercial lot of liquid calf lymph vaccine prepared with the Ikeda virus by multiple pressure on the left upper arm. Two

TABLE II

Local reaction of the monkeys after cutaneous inoculation with smallpox vaccine

Group	Pretreatment with DI's virus	Identity of monkey	Diameters of induration or swelling (mm) at days after smallpox vaccine		
			4	7	9
I	Subcutaneous 1.0 ml ($10^{7.7}$ PFU)	9423	7.5	13.0 (A)	9.0 (A)
		9438	3.0	3.5 (-)	2.0* (-)
II	Intradermal 0.2 ml ($10^{7.0}$ PFU)	9429	6.0	12.0 (-)	10.5**(-)
		9430	4.0	11.0 (B)	7.0**(B)
		9431	6.0	16.5 (-)	11.0**(-)
III	Intramuscular 1.0 ml ($10^{7.7}$ PFU)	9425	6.5	12.0 (A)	8.0**(B)
		9427	6.0	10.0 (-)	7.0**(-)
		9428	6.0	10.5 (B)	7.5**(-)
IV	None (room mate)	9433	5.0	8.5 (A)	11.0 (A)
		9435	5.0	12.5 (-)	19.0 (-)
		9436	6.0	13.5 (B)	11.0 (B)
V	None (fresh)	8887	5.5	12.0 (A)	11.0 (A)
		8988	7.0	15.0 (B)	10.5 (A)

(A): Swelling of an axillary lymph node of a sparrow- to quail-egg size.

(B): Swelling of an axillary lymph node of a bean to soybean size.

(-): No swelling of axillary lymph node.

* : Skin lesion almost completely cured.

** : Slight flat swelling without induration.

[Cited from the previous publication (Tagaya *et al.*, 1973) by the permission of the publisher.]

fresh monkeys were also inoculated as additional controls. The summary of the local reactions of each monkey having appeared in a previous report (Tagaya *et al.*, 1973) is cited here with the permission of the publisher (Table II). All the animals showed erythema and induration quite similar to those observed after the human primary vaccination. However, some of the animals immunized with the DI's virus appeared to show the accelerated wane of local reactions with an earlier scab formation 9 days p.i. compared with the control room mates or fresh animals. Some of the monkeys showed swelling of the axillary lymph node. The results indicated that the immunization of monkeys with the DI's virus did not induce skin resistance strong enough to modify the local reactions to percutaneous inoculation with the ordinary smallpox vaccine 13 weeks later.

The HI antibody response of the monkeys is shown in Fig. 5. By immunization with the DI's virus all the animals responded with a rise in circulating HI antibody, attaining the highest titer 2 to 3 weeks p.i. By percutaneous inoculation with the calf lymph vaccine the antibody rose again, reaching a peak 2 weeks p.i. The time course of the HI antibody response after the challenge with the Ikeda virus was accelerated in animals immunized with the DI's virus compared with the control animals,

X: SACRIFICED

Fig. 5. HI antibody response of cynomolgus monkeys after inoculation of the DIs and Ikeda viruses.

TABLE III
Vaccinia-neutralizing activity of the fresh and heat-inactivated serum of monkeys after immunization with the DI virus and/or challenge with the Ikeda virus

Monkey group	Monkey number and serum code	Preimmunization	Weeks postimmunization (weeks postinoculation with the Ikeda virus)											
			3	7	13(0)	14(1)	15(2)	17(4)	19(6)	26(13)	35(22)	44(31)		
Group I	9423	fresh	<25	ND	ND	365	630	253	ND	92	<25	<25		
		inactivated	<10	<10	<10	63	550	83	ND	<10	<10	<10		
	9438	fresh	<25	ND	ND	63	162	50	ND	54	50	50		
		inactivated	<10	<10	<10	13	173	33	ND	<10	<10	<10		
Group II	9429	fresh	<25	ND	ND	80	365	191	ND	119	92	73		
		inactivated	<10	<10	<10	<10	125	119	ND	<10	<10	<10		
	9430	fresh	35	ND	ND	80	607	253	222	sacrificed				
		inactivated	<10	<10	<10	<10	375	45	27					
Group IV	9431	fresh	50	ND	ND	290	228	167	ND	70	57	122		
		inactivated	<10	<10	<10	28	146	43	ND	12	<10	20		
	9433	fresh	34	ND	ND	42	125	162	47	sacrificed				
		inactivated	<10	<10	<10	<10	28	<10	<10					
Group IV	9435	fresh	34	ND	ND	<25	173	375	149	sacrificed				
		inactivated	<10	<10	<10	<10	29	<10	11					
	9436	fresh	29	ND	ND	<25	62	400	110	sacrificed				
		inactivated	<10	<10	<10	<10	38	<10	13					

as shown by the significant rise in the antibody one week p.i., when no antibody was detectable in the control animals.

Neutralizing antibody was determined with portions of the monkey sera and the titers were included in a previous report (Tagaya et al., 1973). The test was completed later with all the specimens and some corrections were also made by replicate tests. Circulating neutralizing antibody was not detectable in the monkeys given only one shot of the DIs virus, but after challenge with the Ikeda virus a rapid rise was observed, reaching the peak 2 weeks later with a rather rapid decline thereafter. One week after the challenge, five of the eight immunized monkeys showed signifi-

Fig. 6. Vaccinia virus neutralizing activity of the fresh and heat-inactivated sera of monkeys after intramuscular inoculation of the DIs virus and percutaneous challenge with the Ikeda virus.

Fig. 7. Vaccinia virus-neutralizing activity of the fresh and heat-inactivated sera of monkeys after percutaneous inoculation of the Ikeda virus.

cantly increased antibody, while none of the control animals did (Table III, Figs. 6 and 7). The high titers of neutralizing antibody induced in the immunized animals by the challenge with the calf lymph vaccine, however, did not last long but declined rather rapidly. One monkey of the intramuscular immunization group responded poorly with a low titer of the antibody only in the serum collected 2 weeks after the challenge (Fig. 6). The neutralizing antibody response of the control animals was generally low (Table III, Fig. 7).

Another interesting finding was obtained with regard to the dynamics of thermolabile inhibitor of vaccinia virus in the serum of monkeys by inoculation with the DIs virus and challenge with the Ikeda virus. It has been found in our laboratory that some of fresh sera of cynomolgus monkeys reduced the plaque number by vaccinia virus in CE cultures, when mixed with virus and incubated before inoculation as in the neutralization test. In the present experiment neutralizing activities of both fresh and heat-inactivated sera were titrated in parallel. The dynamics of thermolabile and thermostable neutralizing activities in the serum of the monkeys of Group III (im immunization) and Group V (non-immune) are shown in Figs. 6 and 7, as these animals were followed for longer periods. The data on the sera of the animals of the other groups are summarized in Table III. By im inoculation of the DIs virus, circulating thermolabile neutralizing activity increased in all three monkeys, although one of them had the same activity of lower titer in the preimmunization serum. However, thermostable neutralizing antibody was not detectable in these sera before challenge with the Ikeda virus. By percutaneous inoculation with the Ikeda virus

both activities increased rapidly as shown in Fig. 6. The rise in thermolabile as well as thermostable neutralizing activities was also observed in the serum of control nonimmune monkeys after inoculation with the Ikeda virus, and thermolabile neutralizing activity roughly paralleled thermostable neutralizing antibody, but the former appeared earlier and seemed to last longer than the latter. Appearance of neutralizing antibody in this group of monkeys was irregular and the titer generally low (Table III, Fig. 7). The specificity and immunological role of this thermolabile vaccinia virus-neutralizing factor in the serum must await further study. The present observation appears to suggest that some sort of immune status, as shown by the antibody response to the challenge with the Ikeda virus, was induced by one shot of the DIs virus, although circulating neutralizing antibody did not reach a detectable level.

DISCUSSION

Extremely low pathogenicity of the DIs virus in rabbits, mice, guinea pigs and chicks or chickens was established as reported in a previous publication (Tagaya, Kitamura and Sano, 1961). The low virulence of the virus was confirmed also in cynomolgus monkeys by iv, im, sc and id inoculations (Tagaya et al., 1973). Intracerebral inoculation, however, induced in some monkeys mild clinical symptoms as reported previously (Tagaya et al., 1973), although far milder than those induced by other strains of vaccinia virus. When monkeys were sacrificed 10 days p.i., recovery of virus from the brain and spinal cord was not successful. Histologically, however, leptomeningitis purulenta acuta was observed, which may have accounted for manifestation of the clinical symptoms. This may indicate that the DIs virus multiplied to some extent in the leptomeninges near the inoculation site, but some defence mechanism including immune response and interferon production as well as low replicability of the virus in primate cells as suggested by *in vitro* studies (Tagaya et al., 1973) interrupted the multiplication of the virus. On the contrary, the other strains of vaccinia virus examined, including those available for human vaccination, killed the animals within a few days p.i., as reported in previous publications (Aldershoff, Pondman and Pot, 1929; Hurst and Fairbrother, 1930; McIntosh and Scarff, 1930; Eckstein, 1931). This was confirmed by extensive studies, of which a preliminary report appeared elsewhere (Hashizume et al., 1973).

Immunization of monkeys with a single injection of the DIs virus by the sc, id or im route did not induce skin resistance to challenge by the calf lymph smallpox vaccine of the Ikeda strain, although local reactions appeared to be accelerated to some extent compared with those in non-immune animals (Tagaya et al., 1973). HI antibody response was observed, but circulating neutralizing antibody was not detectable by DIs immunization. By challenge with the Ikeda virus, however, immunized animals responded with a rapid rise in both HI and neutralizing antibodies, which reached the peak 2 weeks after challenge. This may suggest that the DIs virus induced a certain degree of basic immunity to poxvirus infection, though not strong enough to prevent or modify skin reaction by the Ikeda virus. Before the applicability of the DIs virus to human vaccination is assessed, more studies are necessary to improve its immunity-inducing capacity in primates, although it is considered as one of the safest vaccinia virus to man from the results of intracerebral inoculation

into monkeys.

The presence of a thermolabile vaccinia virus-neutralizing factor in the serum of some monkeys and the appearance or increase of this activity by the inoculation of the DIs virus or Ikeda virus appear to provide a new field of study. The nature of the thermolabile inhibitor of variola virus in normal guinea pig or rabbit sera has recently been studied by Kitamura and his associates (Kitamura, Miyagawa and Tanaka, 1973; Kitamura, Tanaka and Sugane, 1973), and they also have confirmed that this inhibitor increased after infection with vaccinia virus (personal communication).

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