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Experience in the Study of a Live Vaccine Made from the TP-21 Strain of Malayan Langat Virus*

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The reaction-causing properties and immunogenicity of 2 cloned variants of the Malayan tick virus, Langat TP-21 strain, were studied. One clone, which was not pathogenic for rhesus monkeys when inoculated intracerebrally, caused the formation of antibody in moderate titre against tick-borne encephalitis virus in over 70 % of persons given 2 inoculations of a live vaccine prepared from it. The other variant, which was pathogenic for rhesus monkeys, was characterized by greater immunogenicity but when given as a live vaccine caused acutely febrile reactions and other side-effects in 10 % of inoculated persons 6 to 8 days after administration.

In persons vaccinated with the avirulent vaccine, the antibodies were maintained for over 3 years compared with only 1 year after administration of a killed vaccine. The live vaccine made from the non-pathogenic clone of the Langat virus proved to be suitable for revaccinating patients inoculated with the killed vaccine. The vaccine prepared from the non-pathogenic clone could be more extensively employed for the prophylaxis of tick-borne encephalitis.

The high degree of genetic stability of the tick-borne encephalitis virus makes it extremely difficult to obtain by experimental means attenuated variants of the virus from which to develop a live vaccine that will be more effective than the existing formol-killed vaccine.

The Malayan virus, isolated in 1956 by Dr C. E. Gordon Smith (Smith, 1965), apparently does not cause illness in human beings in natural foci of infection and has close antigenic affinities with the tick-borne encephalitis virus. In view of this, it is of value to try to use the Malayan virus for active immunization against tick-borne encephalitis. Unlike other representatives of the tick-borne encephalitis group, the Langat TP-21 virus is not pathogenic for white mice when administered intraperitoneally or subcutaneously nor for rhesus monkeys when administered intracerebrally. The virus is difficult to reproduce in chick-embryo fibroblast cultures at 40°C and fails to multiply at all at 41.5°C. Passages through such cultures lead to a swift intensification of pathogenicity for mice and monkeys, with a simultaneous rise in the temperature threshold for reproduction in tissue cultures. This variability is due to the selection of the pathogenic virus particles that are always present in the initial strain (Il'enko et al., 1968).

The present paper shows the results of observations on persons inoculated with pathogenic and non-pathogenic variants of the Malayan virus vaccine.

MATERIALS AND METHODS

Virus

The Langat TP-21 virus was obtained in 1958 from Dr C. E. Gordon Smith. Both pathogenic and non-pathogenic clones of the virus were used in the study, the clones having been selected in this laboratory in chick-embryo fibroblast cultures.

Vaccine

The vaccine made from the clone which was non-pathogenic for monkeys was used in two forms—the mouse-brain and tissue-culture forms. Only a tissue-culture vaccine was prepared from the pathogenic clone.

The mouse-brain vaccine was made from the brains of suckling mice infected with the non-pathogenic clone of the Malayan Langat virus and sacrificed when symptoms of disease had

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appeared in 50% of the inoculated animals. The brain suspension was prepared in physiological saline, centrifuged for 30 minutes at 6000 rev/min to 8000 rev/min and checked for specificity, sterility and lack of harmful effects.

The tissue-culture vaccine was prepared in a monolayer culture of chick-embryo fibroblasts. Trypsinized cells of chick embryos 9–12 days old were put into Roux bottles at the rate of 600 000–800 000 per 1 ml of medium and after incubation for 24 hours at 36°C the culture was infected with Langat virus. A brain suspension of the virus, kept in the frozen state at −30°C and previously tested in accordance with the instructions, was usually used for infection. After incubation for 3–4 days at 36°C, the culture fluid was pipetted off, filtered through a thick layer of compressed cotton-wool and then was checked for virus content, specificity and lack of harmful effects. The medium for fibroblast growth consisted of Gey's salt solution with the addition of 5% of bovine serum inactivated by heating at 56°C and previously checked to ensure that it would not inhibit the biological activity of the virus. The maintenance medium was the growth medium as described diluted with an equal quantity of 199 medium.

Vaccination

A 1-ml quantity of vaccine was administered to volunteer subjects subcutaneously twice at a weekly interval in the case of the non-pathogenic virus and once in the case of the pathogenic strain. The formol-killed tissue-culture vaccine made from the diphasic meningoencephalitis virus (Absettarov strain) was administered 3 times as a control.

Blood was taken from the ulnar vein of vaccinated persons before and after vaccination. The serum was heated for 20 minutes at 56°C and stored at 2°C–4°C for subsequent examination.

The neutralization test was carried out in white mice with the Absettarov strain of diphasic meningoencephalitis virus, a mixture of the virus and the test serum being administered intraperitoneally to mice. The results were read on the fourteenth day.

In order to study the reaction-causing properties of the vaccine, temperatures were taken twice daily and the general condition of the vaccinated person was examined (complaints of fatigue readily brought on, headache, loss of appetite, insomnia, etc., were noted). Neuropathologists took part in the clinical examination of the vaccinated persons.

RESULTS

The possibility of using the Malayan Langat virus as a live vaccine against tick-borne encephalitis was studied in cautious and gradually expanding trials with somatically healthy persons aged from 19 to 40 years. The tests were begun with a group of volunteers from the staff of the Department of Virology and medical workers from other establishments.

Inoculations of 2 preparations made from a non-pathogenic clone (mouse-brain and tissue-culture vaccines) were given. When it was found that the vaccine was harmless but only moderately immunogenic, limited trials were carried out with a tissue-culture vaccine prepared from a clone pathogenic to monkeys which was isolated after 5–10 passages through a culture of chick-embryo fibroblasts.

The basic characteristics differentiating the pathogenic and non-pathogenic clones isolated from the original strain of the Langat virus have already been described (see Table 5, Il'enko et al., 1968).

Vaccines tested were evaluated on the basis of the following indices: lack of harmful effects, the development of viraemia, immunogenicity and the length of time for which antibodies could still be found in the blood of vaccinated persons.

Study of the vaccine made from the non-pathogenic clone of the Langat virus

Reaction-causing properties. Study of the reaction-causing properties of the live Langat virus vaccine was carried out in observations on human beings who had been given various doses of the virus (from 10 to 10^7 LD_{50} for mice). In a group of 1113 persons vaccinated with mouse-brain and tissue-culture vaccines made from the non-pathogenic clone, no reactions were observed, except a few cases of cutaneous hyperaemia on the site of the injection of the mouse-brain vaccine. Among 64 persons given the tissue-culture vaccine orally no reactions of any sort were noted (Table 1).

Development of viraemia in the vaccinated persons. One of the signs indicating the intensity of virus multiplication in vaccinated persons is the development of viraemia. To measure this, the blood of vaccinated people from 1 to 15 days after a single administration of vaccine was examined. Blood taken from the ulnar vein was injected into the brain of white mice; the results were considered

TABLE 1

RESULTS OBTAINED FROM A STUDY OF CLINICAL REACTIONS IN PERSONS INOCULATED WITH THE LIVE VACCINE MADE FROM THE NON-PATHOGENIC CLONE OF THE LANGAT VIRUS

Vaccine	Route of administration	No. of persons inoculated	Reactions noted	
			Rise in temperature	Other reactions
Mouse brain	Subcutaneous	610	—	—
Tissue culture	Subcutaneous	503	—	—
	Oral	64	—	—
Total		1 177	—	—

negative after 2 blind passages through the brain of the inoculated animals.

Out of a total of 50 persons to whom large doses of virus were administered (over 7.0 log LD_{50} per vaccination) the virus was successfully isolated from the blood of 3 (in 1 case after a single extra passage and in 2 cases after 2 extra passages). The virus was isolated only 24 hours after vaccination; later than that there was no viraemia. These cases of early discovery of virus are apparently due to the survival of the virus administered and not to its multiplication in the organism. In 10 persons who had been given the vaccine orally, it proved impossible to isolate virus from the blood (Table 2).

TABLE 2

FREQUENCY OF VIRAEMIA IN PERSONS INOCULATED WITH THE LIVE NON-PATHOGENIC LANGAT VIRUS

Route of administration	No. of persons examined	No. of persons with viraemia when examined on the following number of days after administration of the vaccine:					
		1	3	5	6–8	9–10	12–15
Subcutaneous	50	3	—	—	—	—	—
Oral	10	—	—	—	—	—	—

Immunogenicity of the vaccine. A single administration of avirulent vaccine had a rather low immunizing effect and for that reason most of our observations deal with persons vaccinated twice.

In the first tests (Table 3) sera from vaccinated

TABLE 3

RESULTS OF PARALLEL EXAMINATIONS OF SERA FROM PERSONS INOCULATED WITH TP-21 IN A NEUTRALIZATION TEST WITH DIPHASIC MENINGOENCEPHALITIS AND TP-21 VIRUSES

Serial No. of serum	Neutralizing doses of virus when the sera interact with the virus indicated (expressed as $\log_{10}$ values)			
	Diphasic meningoencephalitis		TP-21	
	Before vaccination	After vaccination	Before vaccination	After vaccination
1	0	2.4	0	2.0
2	0.5	2.4	0	2.0
3	2.4	2.4	2.4	2.4
4	0	2.5	0	2.4
5	0	2.5	0	2.4
6	0	2.7	0	3.7
7	0	3.0	0	3.7
8	1.5	3.0	1.5	2.7
9	1.0	3.2	1.0	3.5
10	0	3.2	0	4.0
11	0	3.4	0	4.0
12	2.0	3.4	0.5	4.0
13	0	4.0	0	4.0
14	0	4.0	0	4.5
15	0	4.0	0	4.7
16	0	4.5	0	4.7
17	0	5.0	1.5	4.0

TABLE 4
FREQUENCY AND DATES OF APPEARANCE OF ANTIBODIES IN THE BLOOD OF PERSONS VACCINATED WITH VARIOUS VACCINES MADE FROM LANGAT VIRUS

Vaccine	Method of preparation	Route of administration	No. of persons examined	No. of those in which the neutralization test was positive				
				Absolute value	%	After 1 month	After 2 months	After 3 months
Killed	Tissue culture	Subcutaneous	148	124	84	NI	124	NI
Live	Mouse brain	Subcutaneous	77	67	87	67	67	67
	Tissue culture	Subcutaneous	66	46	70	35	46	46
		Oral	64	35	55	15	28	35

[a] NI = Not investigated.

persons were tested in parallel by means of the neutralization test with 2 different viruses, the Langat virus and the Absettarov strain of diphasic meningoencephalitis. Since the results of these comparative investigations showed quite close agreement, subsequent neutralization tests were carried out only with the Absettarov strain of the diphasic meningoencephalitis virus. The test was made by means of intraperitoneal infection of mice with mixtures of serum and virus.

Table 4 shows the results of neutralization tests with the sera of persons to whom mouse-brain and tissue-culture vaccines had been administered. The immunogenicity of the live mouse-brain vaccine made from a non-pathogenic strain approached that of the formol-killed vaccine. The live tissue-culture vaccine proved less immunogenic, a feature that was probably due to the fact that it contained a smaller quantity of active virus.

The smallest immunizing effect was obtained in a group of persons to whom the tissue-culture vaccine had been administered orally. It is also interesting to note that antibodies formed more slowly in persons given the tissue-culture vaccine. (This also could be ascribed to the smaller dose of virus administered.) While in the group of those who were given the mouse-brain vaccine positive results in the neutralization test occurred after 1 month and afterwards remained unchanged in regard to titre, the same process took 2 months in the group given tissue-culture vaccine and in those given the vaccine orally as much as 3 months, when the greatest number of persons with antibodies in the blood was recorded.

Table 5 shows the effect of the dose of virus administered on the frequency of rises in antibody titre. These data obviously explain the advantage of vaccination with the mouse-brain vaccine which contained 2.5–3.5 log LD_{50} as much virus as the tissue-culture vaccine (Table 5).

TABLE 5
EFFECT OF THE DOSE OF VIRUS ADMINISTERED ON THE NUMBER OF POSITIVE FINDINGS IN THE NEUTRALIZATION TEST AMONG VACCINATED PERSONS

Amount of virus administered to persons vaccinated (expressed as log LD_{50}/1 ml)	Percentage of persons responding to vaccine with a rise in antibody level
6.5	80
4.5	66
2.5	43
0.5–1.0	6

Table 6 shows comparative results of the neutralization test when people were immunized with mouse-brain or tissue-culture vaccines of various types (killed or live) by the subcutaneous or oral routes.

Duration of immunity. Although examination of the patients concerned 6 weeks after administration of the live vaccine made from the non-pathogenic clone of the Langat virus failed to show any substantial advantages over the formol-killed vaccine, when humoral immunity was tested at later dates the superiority of the live vaccine was clearly shown.

The main disadvantage of the killed vaccine is the need for annual revaccination of considerable groups of persons who run a risk of contracting

TABLE 6

IMMUNOGENICITY OF THE KILLED AND LIVE VACCINES MADE FROM TICK-BORNE ENCEPHALITIS VIRUS AND THE TP-21 STRAIN OF LANGAT VIRUS ACCORDING TO THE FINDINGS IN THE NEUTRALIZATION TEST WITH SERA TAKEN FROM VACCINATED PERSONS 6 WEEKS AFTER VACCINATION [a]

Vaccine	Method of preparation	Route of administration	No. of inoculations (of 0.25 ml)	No. of persons examined	No. of those with positive findings in the neutralization test		Neutralization indices (expressed as $\log_{10}$ values)			
					Absolute value	%	Negative (0–2.0)	Low (2.1–3.0)	Medium (3.1–5.0)	High (≥5.1)
Killed	Tissue culture	Subcutaneous	3	148	124	84	16	11	26	47
Live	Mouse brain	Subcutaneous	2	76	67	87	12	22	20	46
	Tissue culture	Subcutaneous	2	66	46	70	30	45	25	0
		Oral	2	64	35	55	46	50	4	0

[a] Expressed as percentages.

tick-borne encephalitis. Meanwhile, our observations of persons inoculated with the live Langat virus vaccine showed that immunity was maintained for at least 3 years (Table 7). In that time the sera of most persons inoculated with the killed vaccine had lost their virus-neutralizing antibodies, whereas humoral immunity was well maintained in the group vaccinated with the live vaccine.

Study of the vaccine prepared from the pathogenic clone of the Langat virus

In view of the only moderate immunogenicity of the live vaccine made from the non-pathogenic clone of the Langat virus, a preparation made from a clone that was more pathogenic to monkeys (see Table 5, Il'enko et al., 1968) was tested.

TABLE 7

CHANGES IN HUMORAL IMMUNITY 3 YEARS AFTER INOCULATION IN PERSONS IN GROUPS INOCULATED WITH THE KILLED VACCINE MADE FROM DIPHASIC MENINGOENCEPHALITIS VIRUS OR WITH LIVE VACCINE MADE FROM TP-21 STRAIN OF LANGAT VIRUS AND WHO HAD ANTIBODIES 6 WEEKS AFTER INOCULATION

Vaccine	No. of inoculations (of 0.25 ml)	Amount of virus per inoculation (expressed as log LD_{50})	No. of persons examined	Time between inoculation and examination	Neutralization index (expressed as $\log_{10}$ values)			
					Negative (0–2.0)	Low (2.1–3.0)	Medium (3.1–5.0)	High (≥ 5.1)
Killed tissue-culture vaccine made from diphasic meningoencephalitis virus	3	9.0	24	6 weeks	—	—	2	22
				3 years	17	6	1	—
Live mouse-brain vaccine made from avirulent Langat TP-21 virus	2	9.0	34	6 weeks	—	10	9	15
				3 years	4	14	11	5
Live tissue culture vaccine made from non-pathogenic Langat TP-21 virus	2	5.5–6.5	23	6 weeks	—	8	15	—
				3 years	6	6	11	—

TABLE 8

FREQUENCY OF CLINICAL REACTIONS AND VIRAEMIA IN RESPONSE TO THE ADMINISTRATION OF A LIVE VACCINE MADE FROM A LANGAT VIRUS CLONE PATHOGENIC FOR MONKEYS

No. of persons inoculated	Clinical reactions			Persons examined for viraemia	No. of persons with viraemia the following number of days after inoculation:					
	Rise in body temperature	Other symptoms			1	3	5	6–8	9–10	12–15
		Total	Development of a meningeal syndrome							
101	10	4	2	39	—	1	1	28	3	—

Administration of the pathogenic clone caused viraemia to develop in over 70% of those inoculated on the sixth to eighth days after inoculation and in a number of cases clinical side-effects appeared—a rise in temperature and, in 2 cases out of 101 persons inoculated, the development of a mild meningeal syndrome (Table 8).

In 97% of the persons inoculated with the vaccine made from the pathogenic clone there was an intensive rise in the level of virus-neutralizing antibodies, with a predominance of medium- and high-neutralization indices (Table 9), when the serum neutralized 10^3–10^5 LD_{50} infective doses of tick-borne encephalitis virus. In those vaccinated, humoral immunity was maintained very solidly in the 12–18 months following inoculation. Nevertheless, in view of the proven possibility of a meningeal syndrome developing in those inoculated with the more pathogenic clone of the Langat virus, work with the preparation was stopped and further researches were carried out only with the non-pathogenic clone.

CONCLUSION

These trials, covering 1177 persons, of the reaction-causing and immunogenic properties of live vaccine prepared from a clone of the Langat virus which was non-pathogenic for monkeys have established that the vaccine is harmless and adequately immunogenic. Virus-neutralizing antibodies accumulated in the blood of over 70% of the persons inoculated and were maintained during 3 years of observation.

A clone of the Langat virus which was pathogenic for monkeys caused quite serious clinical reactions in some persons who were inoculated with it. The use of this clone as a live vaccine is, therefore, not possible in practice.

TABLE 9

IMMUNOGENICITY OF KILLED AND LIVE VACCINES MADE FROM TICK-BORNE ENCEPHALITIS VIRUS AND A PATHOGENIC CLONE OF LANGAT VIRUS ACCORDING TO THE NEUTRALIZATION TEST WITH SERA TAKEN FROM INOCULATED PERSONS 6 WEEKS AFTER INOCULATION [a]

Vaccine	Method of preparation	Route of administration	No. of inoculations	No. of persons examined	No. of those with positive findings in the neutralization test		Neutralization indices (expressed as $\log_{10}$ values)			
					Absolute value	%	Negative (0–2.0)	Low (2.1–3.0)	Medium (3.1–5.0)	High (≥5.1)
Killed	Tissue culture	Subcutaneous	3	148	124	84	16	11	26	47
Live	Tissue culture	Subcutaneous	1	62	60	97	3	3	32	62

[a] Expressed as percentages.

RÉSUMÉ

Dans un article précédent, les auteurs ont montré que par passages du virus Langat TP21 sur cultures cellulaires de fibroblastes d'embryon de poulet on pouvait sélectionner des clones pathogènes et non pathogènes pour le singe rhésus et la souris. Des vaccins vivants préparés avec l'un et l'autre de ces deux variants ont été expérimentés chez l'homme.

Le vaccin vivant à base de virus Langat non pathogène a été administré par voie sous-cutanée à 1113 volontaires âgés de 19 à 40 ans. Aucune réaction secondaire importante n'a été observée. En raison du faible pouvoir immunogène d'une dose vaccinale unique, la plupart des sujets ont reçu une 2e injection. Il en est résulté dans 70% des cas une élévation nette du titre des anticorps sériques neutralisant le virus qui s'est maintenue pendant 3 ans (alors que le vaccin tué utilisé couramment ne protège que pendant un an). Un essai de vaccination par voie orale portant sur 64 personnes a montré que si ce mode d'administration est praticable, l'immunité obtenue est de faible niveau.

L'essai de vaccin à base de virus pathogène a été effectué sur 101 volontaires qui ont reçu, par voie sous-cutanée, une dose unique de la préparation. Chez 97% des vaccinés, on a constaté une hausse considérable du titre des anticorps neutralisants, persistant pendant 12 à 18 mois. Cependant l'apparition de réactions secondaires (fièvre: 10 cas; réaction méningée: 2 cas) a conduit à interrompre les essais.

Si l'utilisation pratique du vaccin Langat à base de virus pathogène ne peut être envisagée, il semble en revanche que le vaccin vivant préparé à partir du variant avirulent puisse, en raison de son innocuité et de son pouvoir immunogène satisfaisant, être employé avec profit pour la vaccination contre l'encéphalite à tiques.

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