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Inoculation of hamsters with a temperature sensitive (ts) mutant of parainfluenza 3 virus*

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SUMMARY

The multiplication pattern of a temperature-sensitive (ts) mutant of bovine parainfluenza 3 virus was studied in hamsters and compared with that of a virulent virus strain. The ts mutant was recovered regularly from the nasal mucosa and not from the lungs, whereas the virulent virus multiplied in the lungs as well as in the nasal mucosa.

The serological response induced by the mutant was comparable to that obtained after inoculation of the virulent virus.

This ts mutant may be a potential candidate for a live intranasal vaccine against bovine parainfluenza 3 infection.

INTRODUCTION

Local antibodies in the respiratory mucosa play an important role in the immunity against respiratory virus infections. The formation of such local antibodies can be induced by the intranasal administration of live, attenuated viruses which multiply locally in the nasopharyngeal mucosa. Theoretically, the safety margin of this procedure could be greatly increased by the use of viruses which have lost their capacity to multiply in the lower respiratory tract but are still capable of multiplying in the nasal mucosa. The difference which exists between the normal temperature of the nasal mucosa and that of the lungs offers an attractive approach to this problem. This approach has been investigated recently in studies in laboratory animals with temperature-sensitive (ts) mutants of various respiratory viruses (Gharpure, Wright & Chanock, 1969; Mackenzie, 1969; Mills, van Kirk, Hill & Chanock, 1969). The hamster has served as an experimental model in several of these studies (Mills *et al.* 1969; Potash *et al.* 1970; Wright, Woodend & Chanock, 1970).

In the present study we inoculated hamsters with a ts mutant of parainfluenza 3 virus of bovine origin and studied the behaviour of this mutant in comparison with that of a wild strain of the same virus.

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MATERIALS AND METHODS

Viruses

R.L.B. 103. This ts mutant was isolated in our laboratory from the 23B variant of the Umeå strain (Bakos & Dinter, 1960) of parainfluenza 3 virus; this strain was obtained originally from Dr Z. Dinter, Institute of Virology, Uppsala. The ratio of the growth at 30° C. versus the growth at 39° C. for the ts mutant was $\geqslant 10^{5.5}$ TCID 50. The virus used in the present study was grown in primary fetal bovine kidney cell cultures.

Virulent virus: WT strain. This strain was isolated in our laboratory from the lung lymph nodes of a calf with pneumonia. This strain had been passed 5 times in primary bovine kidney cell cultures. The ratio of its growth at 30° C. versus 39° C. was $\leqslant 10^{-1}$.

Cell cultures

Primary cultures of bovine fetal kidney (PBFK) were used throughout the study. The growth medium consisted of Hanks's balanced salt solution with 5% lactalbumin hydrolysate and 10% calf serum. As maintenance medium we used Eagle's medium with 2% virus-screened agamma newborn calf serum (Hyland Laboratories).

Hamsters

Syrian hamsters were obtained from our own breeding colony. They were 7–8 weeks old when used in the study.

Animal inoculation

Each hamster was inoculated intranasally with a volume of 0·1 ml. per nostril. The animals were killed at regular intervals after inoculation; heparinized blood, the lungs and the nasal turbinates were removed at the time of autopsy.

Virus isolation

The presence of virus in the organs was determined as follows: the tissues were ground in a mortar and a 1/10 (w/v) suspension was made in phosphate buffered saline (PBS) containing 200 I.U. penicillin, 200 μg. streptomycin and 100 units nystatin per ml.

The samples were centrifuged for 30 min. at 3000 rev./min. before inoculation; the blood samples were used without further treatment. Tenfold dilutions were made in PBS.

The samples were inoculated onto PBFK culture tubes, using four tubes per dilution and 0·2 ml. per tube. Two tubes were incubated at 30° C. and two others at 39° C. for 7 days. A haemadsorption test using guinea-pig erythrocytes was performed at the end of the observation period.

Table 1. *Isolation of parainfluenza 3 virus from the organs of hamsters killed at 2, 3 and 4 days after inoculation*

		Days after inoculation								
		2			3			4		
			Log titre at			Log titre at			Log titre at	
Group	Organ	Animal no.	30° C.	39° C.	Animal no.	30° C.	39° C.	Animal no.	30° C.	39° C.
A, ts mutant	Nasal mucosa	1	⩾ 2	–	4	2	–	7	⩾ 2	0
		2	0	–	5	2	0	8	2	–
		3	1	–	6	⩾ 2	–	9	2	–
	Lungs	1	–	–	4	–	–	7	–	–
		2	–	–	5	–	–	8	–	–
		3	–	–	6	–	–	9	–	–
	Blood	1	–	–	4	–	–	7	–	–
		2	NT	NT	5	–	–	8	–	–
		3	NT	NT	6	–	–	9	–	–
B, WT strain	Nasal mucosa	10	⩾ 2	⩾ 2	13	⩾ 2	⩾ 2	16	⩾ 2	⩾ 2
		11	0	1	14	⩾ 2	⩾ 2	17	⩾ 2	⩾ 2
		12	⩾ 2	⩾ 2	15	⩾ 2	2	18	⩾ 2	⩾ 2
	Lungs	10	⩾ 2	⩾ 2	13	⩾ 2	⩾ 2	16	⩾ 2	⩾ 2
		11	⩾ 2	⩾ 2	14	⩾ 2	2	17	⩾ 2	⩾ 2
		12	1	–	15	⩾ 2	1	18	⩾ 2	⩾ 2
	Blood	10	–	–	13	–	–	16	–	–
		11	NT	NT	14	–	–	17	–	–
		12	NT	NT	15	–	–	18	–	–

– = negative result. NT = not tested.

Table 2. *Haemagglutination-inhibiting antibody response in hamsters inoculated with parainfluenza 3 virus*

Group	Inoculum	HA antigen used	Days after inoculation: 0 Titre	10 Titre	21 Titre
A	ts	WT	< 10	< 10	320
		ts	< 10	20–40	1280
B	WT	WT	.	40	640–1280
		ts	.	80	1280
C	None controls	WT	< 10	< 10	< 10
		ts	< 10	< 20	< 10

Haemagglutination-inhibition (HI) test

A 0·5 % guinea-pig erythrocyte suspension was used; sera from different animals taken on the same day after inoculation were pooled, inactivated at 56° C. for 30 min., treated first with an equal volume of a 25 % kaolin suspension and then with a 50 % suspension of guinea-pig erythrocytes; 1 volume of this erythrocyte suspension was added to 10 volumes of the 1/2 serum dilution. Four haemagglutinating units were used in a volume of 0·25 ml.

RESULTS

Thirty-six hamsters were divided in three groups. The first group (A) received the ts mutant; the inoculum had a titre of $10^{6\cdot3}$ TCID 50/0·1 ml. at 30° C.; the second group (B) was inoculated with the WT strain; the titre of the inoculum was $10^{7\cdot3}$ TCID 50/0·1 ml. at 37° C. The animals of the third group (C) were used as controls and were given Eagle's basal medium. On days 2, 3 and 4 after inoculation three animals of group A and B each were killed and their organs used for virus isolation attempts. The remaining animals were bled on days 10 and 21 and their serum examined for the presence of HI antibodies.

The results of the virus isolation studies are summarized in Table 1. No viraemia could be detected in any of the animals examined whether they were inoculated with the wild WT virus or with the ts mutant. In both inoculated groups all animals examined on the 2nd, 3rd and 4th days after inoculation harboured virus in their nasal mucosa. In group A inoculated with the ts strain all samples incubated at 30° C. were positive. No definite conclusions can be drawn from the titrations because in most cases no end-point was reached, but there may be some indication that the titre in the animals killed on the 2nd day was somewhat lower than in those killed on the 3rd and 4th days. All cultures inoculated with samples from group A and incubated at 39° C. remained negative, except for two samples where a slight haemadsorption was observed in the cultures inoculated with the undiluted material. In group B no difference was noticed between the cultures incubated at 30° and 39 °C.

Virus was recovered from the lungs of all nine animals which had received the

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wild strain, whereas all attempts to isolate virus from the group inoculated with the ts mutant failed.

The results of the HI tests on the sera are shown in Table 2. Each serum pool was tested against both antigens, WT and the ts strain. No significant differences were observed. No HI antibodies were present in the pre-inoculation sera. The control animals remained seronegative throughout the observation period. In groups A and B low HI titres were demonstrated on the 10th day after inoculation. The titres had increased significantly on day 21. They were comparable in both groups.

DISCUSSION

Ts mutants can be expected to offer a wider margin of safety than viruses attenuated by other means, in those respiratory infections where the pathogenicity of the organism is related to its capacity to multiply in the lungs. In their experiments with an influenza A 2 strain in hamsters, Mills *et. al.* (1969) obtained a strong reduction of the multiplication rate of the ts mutant as opposed to the parent virus. Similar results were obtained by Wright *et. al.* (1970) with ts mutants of respiratory syncytial virus. A ts mutant of parainfluenza 1 virus showed an analogous pattern in a study in hamsters reported by Potash *et al.* (1970).

The results of our study in hamsters with parainfluenza 3 virus confirmed the theoretical expectations. The multiplication of the ts mutant was restricted to the nasal mucosa of the inoculated animals. Although the formation of local antibodies was not checked, the demonstration of circulating antibodies as a result of the intranasal application of the virus may be regarded as an indication that in all probability a local immunity was present. The circulating antibody titres observed in the group which had received the ts mutant were of the same order as in those which had received the wild strain. From the comparison of the recovery rates in cultures incubated at 30° and 39° C. we may conclude that one hamster passage had no apparent effect on the ts properties of the virus.

The behaviour of the wild strain contrasted sharply with that of the ts strain. It could be recovered from the lungs as well as from the nasal mucosa of the inoculated animals.

Live attenuated parainfluenza 3 viruses administered intranasally have been shown by Bögel & Liebelt (1964) and by Gutekunst, Paton & Volenec (1969) to produce immunity in calves. For the immunization against this disease a ts mutant might offer some additional advantages over live vaccines attenuated by other procedures. Experiments in calves, however, are required to check if the ts mutant used in our study behaves in its natural host in the same way as it does in hamsters.

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