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Dissemination and immunogenicity of live TRIC agent in baboons after parenteral injection

II. Experiments with a 'slow-killing' strain

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INTRODUCTION

During serial passage in chick embryos, some strains of trachoma/inclusion conjunctivitis (TRIC) agents undergo mutation and produce variants that, for a given dose, kill chick embryos more rapidly than the parent strain (Reeve & Taverne, 1963). Taverne, Blyth & Reeve (1964) termed these mutants *f*, or 'fast-killing', to distinguish them from the 'slow-killing' parent strains.

As part of a series of experiments with trachoma vaccines, we previously reported that the 'fast-killing' MRC-4 *f* strain of TRIC agent multiplied in baboons after parenteral injection (Collier & Smith, 1967). Subcutaneous injection was followed by multiplication in the skin and regional lymph nodes, and the agent was found in the spleen up to 11 days later. After intravenous injection, it was isolated from the blood up to 18 hr. later; the amount of infective agent in the spleen attained much higher levels and persisted longer than after subcutaneous injection, and in some animals there was good evidence of replication in peripheral lymph nodes. Twenty-four hours after intravenous (but not after subcutaneous) injection, TRIC agent was isolated from the liver. In line with earlier findings (Collier, 1961; Collier & Blyth, 1966), baboons injected intravenously were more resistant to challenge by the conjunctival route than were those inoculated subcutaneously.

In this laboratory, Blyth (1967*a*, *b*) found that the MRC-4 *f* strain was more virulent for guinea-pigs than the parent strain MRC-4 (full designation: TRIC/ /GB/MRC-4/ON) isolated from the eye of a newborn baby (Jones, 1961; Jones & Collier, 1962). After intracutaneous injection, it multiplied in the skin to higher titres and for longer periods than the parent strain, persisted longer in the regional nodes, and, unlike MRC-4, usually appeared in the spleen 3–10 days after injection.

The present paper describes an experiment to determine whether similar differences in the virulence of *s* and *f* strains could be established in baboons and, if so, whether such differences are related to immunogenicity; these questions are obviously important in choosing strains of TRIC agent for vaccine production.

MATERIALS AND METHODS

The procedures and materials were as previously described for our Experiment II (Collier & Smith, 1967) except that the vaccine was prepared from MRC-4.

General plan of experiment

On the first day (D 0) two groups each of 24 young baboons (*Papio cynocephalus*) were inoculated with a single 1·0 ml. dose of the same suspension of live MRC-4 vaccine. The animals of group A were injected subcutaneously into the medial aspect of the right upper arm, and those of group B intravenously into the femoral vein. A third group (C) of seven animals served as uninoculated controls. At intervals after injection (Tables 1 and 2), pairs of animals from groups A and B were killed and the amount of TRIC agent in various tissues was assayed by titration in chick embryo yolk sacs. In our previous experiment, TRIC agent was rarely detectable in small samples of the liver; this organ was therefore not tested. Titres were recorded in terms of $\log_{10}$ ELD 50 (50 % egg lethal dose) recovered from the skin lesion, group of lymph nodes, or whole spleen; the figures for the spleen were estimated from titrations of samples weighing approximately 1 g. The degree of immunity induced by the injections was estimated by comparing the resistance of representatives of the vaccinated and control groups to conjunctival challenge with the homologous strain.

Vaccine

This was prepared from the 9th chick embryo passage of strain MRC-4, partially purified from heavily infected yolk sacs that were harvested when 50 % of the inoculated chick embryos had died. Each sac was shaken for 2 min. at high speed with 5 ml. phosphate-buffered saline (Dulbecco & Vogt, 1954), strained through gauze to remove gross debris, and centrifuged at 2000 rev./min. for 15 min. in an angle head. The supernatant, constituting the 'vaccine' was stored in liquid nitrogen in 0·5 ml. amounts. Its titre was $10^{5\cdot8}$ ELD 50/ml.

Test for immunity

Eleven days after inoculation, the immunity of representatives of groups A and B was tested by inoculating their conjunctivae with the same suspension of MRC-4 used previously (Collier & Smith, 1967) and stored since then in liquid nitrogen. It was prepared from yolk sacs infected with the 8th chick embryo passage of this strain, and had a titre of $10^{5\cdot6}$ ELD 50/ml. The course of infection was compared with that in the control group C challenged similarly. The methods of scoring for severity of physical signs and presence of inclusion bodies were those of Collier & Blyth (1966).

RESULTS

Group A

Skin lesions. Within 48 hr. of subcutaneous injection, papular skin lesions 5–10 mm. in diameter appeared; as judged after dissection, necrosis was minimal. The lesions attained their maximum size on D 2 and regressed rapidly during the

first week (Table 1); by D 11 none were visible. Five minutes after inoculation, just over 10^4 ELD 50 of TRIC agent (i.e. 2 % of the amount inoculated) was recovered from the injection site in each of the two baboons examined at this time (Table 2). At 1 hr., $10^{3\cdot1}$ and $10^{5\cdot9}$ ELD 50 were recovered, although the latter figure is probably an overestimate; in this titration of skin suspension, the results

Table 1. *Weights of whole organs and tissue samples from baboons injected subcutaneously (group A)*

Days after injection	Baboon no.	Weights (g.) Skin lesion	Regional lymph nodes	Spleen
0 (5 min.)	407	0·1	NT	NT
	408	0·5		
0 (1 hr.)	409	0·3	0·6	NT
	410	0·1	0·7	
1 (18 hr.)	411	0·1	0·7	9·0
	412	0·1	0·4	7·7
2	413	0·9	0·8	11·8
	414	1·0	0·3	13·2
4	415	0·4	1·7	15·4
	416	0·1	0·7	9·6
7	417	0·1	2·1	12·2
	418	0·4	0·6	5·7
11	419	0·2	1·9	12·6
	420	0·1	0·6	9·1
15	421	0·4	0·5	8·0
	422	0·3	0·9	12·6
21	423	0·2	0·9	9·2
	424	0·2	0·9	7·5
28	425	0·2	1·2	7·2
	426	0·2	0·8	12·5

NT = Not tested.

Table 2. *Titres of TRIC agent in skin and regional lymph nodes of baboons injected subcutaneously (group A)*

Time after injection	Baboon no.	Skin lesion	Regional lymph nodes
D 0 (5 min.)	407	4·1*	NT
	408	4·2	NT
D 0 (1 hr.)	409	3·1	1·9
	410	5·9	< 1·2
D 1 (18 hr.)	411	< 1·9	< 0·8
	412	< 0·2	< 1·1
D 2 (42 hr.)	413	3·7	< 2·1
	414	3·2	< 1·7

* Infectivity titres expressed as $\log_{10}$ ELD 50 contained in the whole skin lesion or group of lymph nodes. NT = Not tested.

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suggest carry-over of particulate material through the dilution series, with an abnormally high proportion of specific deaths at the higher dilutions. At 18 hr., no infective agent was isolated from either of the skin lesions tested. (In Tables 2 and 4, negative results are given in terms of the minimum infective titre detectable, taking into account the ratio of the weight of tissue inoculated into chick embryos to the total weight of the tissue or organ, and the lowest dilution tested.) The negative findings at 18 hr. are similar to those with MRC-4 *f* (Collier & Smith, 1967). Infective TRIC agent reappeared in the skin on D 2, but we were unable to detect it thereafter.

Regional lymph nodes. Apart from an occasional animal in whom the right axillary nodes appeared somewhat congested, the nodes draining the inoculation site remained virtually normal to the naked eye. Table 1 shows that in some animals increases in weight were observed from D 4 until the end of the experiment on D 28. Infective agent was recovered in low titre from the regional nodes of one animal an hour after injection, but could not be detected thereafter.

Spleen. In nearly half the animals examined there was an increase in spleen weight from D 2 onwards (Table 1); but TRIC agent could not be isolated from spleens tested at intervals from 18 hr. to 28 days after injection.

Blood. The agent was not isolated from blood samples taken 1 hr. and 18 hr. after subcutaneous injection.

Table 3. *Weights of whole organs and tissue samples from baboons injected intravenously (group B)*

Days after injection	Baboon no.	Weights (g.) Axillary lymph nodes	Spleen
1	391	0·5	9·2
(18 hr.)	392	0·6	10·1
2	393	0·6	7·3
	394	0·6	9·3
4	395	0·6	10·3
	396	0·8	11·4
7	397	0·6	9·8
	398	1·0	10·2
11	399	0·4	9·4
	400	1·0	14·0
15	401	1·0	15·8
	402	0·8	6·3
21	403	0·6	15·1
	404	1·1	11·9
28	405	1·0	11·8
	406	0·6	8·8

Group B

Lymph nodes. In about half the baboons examined from D 1 to D 28, the nodes in both axillae were hyperaemic. In several animals there was a moderate increase in the weight of this group of nodes from D 7 onwards (Table 3). TRIC agent could

not be isolated on first testing at 18 hr. (Table 4). It was detected in one of the pair examined at 42 hr., but all subsequent tests gave negative results.

Spleen. There was a moderate increase in average weight, first apparent on D11 and reaching a maximum on D21 (Table 3). TRIC agent could not be detected in spleens tested at 18 hr. On D2, but not thereafter, the agent was isolated from the spleens of both baboons tested (Table 4).

Blood. As with the animals inoculated subcutaneously, the agent was not detected in blood samples tested 1 hr. and 18 hr. after injection.

Table 4. *Titres of TRIC agent in spleen and peripheral lymph nodes of baboons injected intravenously (group B)*

Time after injection	Baboon no.	Lymph nodes	Spleen
D1	391	< 0·9*	< 2·3
(18 hr.)	392	< 1·0	< 2·4
D2	393	2·3	2·7
(42 hr.)	394	< 2·0	3·3

* Infectivity titres expressed as $\log_{10}$ ELD50 contained in the whole group of lymph nodes or whole spleen.

Table 5. *Response to conjunctival challenge in terms of individual and group scores*

Group	Baboon no.	Cumulative score at 28 days after challenge*	Mean score for group
A (subcutaneous)	435	37	34·0
	436	48	
	437	21	
	438	25	
	439	26	
	440	47	
B (intravenous)	429	23	31·0
	430	30	
	431	50	
	432	40	
	433	17	
	434	26	
C (controls)	441	45	43·1
	442	45	
	443	62	
	444	40	
	445	35	
	446	29	
	447	46	

* The individual cumulative scores are the sums of the scores recorded at four examinations made at weekly intervals after challenge.

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Immunity to conjunctival challenge

Eleven days after parenteral injection the immunity of representatives of groups A and B was tested by comparing the severity of ophthalmic infection with that in control group C after conjunctival challenge with the homologous strain. Although the vaccinated animals had somewhat lower scores than the controls in terms of severity of physical signs and presence of inclusions in conjunctival scrapings (Table 5), analysis of variance showed that these differences were not statistically significant.

DISCUSSION

In our first paper on this subject (Collier & Smith, 1967) we described two experiments, the second of which dealt with the effects of single subcutaneous or intravenous injections of MRC-4 *f*, and is referred to in the Introduction. This experiment was closely comparable with that on the parent 'slow-killing' strains reported here, with one important difference to which attention must be drawn. Because of their ability to multiply to high titres in chick embryos 'fast-killing' strains such as MRC-4 *f* are potentially important in vaccine production; and the earlier investigation was specifically designed to determine whether a dose similar to that used for an actual vaccine would multiply in primate hosts after parenteral injection. The titre of the experimental MRC-4 *f* vaccine was $10^{7 \cdot 1}$ ELD 50/ml.; but the vaccine made from MRC-4 contained 20 times less infective agent.

With this reservation, there was a pronounced difference between the behaviour of MRC-4 and its 'fast-killing' variant. After a subcutaneous dose, MRC-4 *f* multiplied to high titre in the injection site and could still be found there up to 3 weeks later; it also multiplied readily in the regional nodes, and appeared in the spleen 4–11 days after injection; by contrast, MRC-4 multiplied only to a limited extent in the skin, and could not be recovered after D 2. That some multiplication did take place can be inferred from the loss of infectivity at 18 hr., and its reappearance at 42 hr.; the timing of these events, which were also observed with MRC-4 *f* in baboons, and with MRC-4 and MRC-4 *f* in guinea-pigs (Blyth, 1967*b*) accords with that of a single multiplication cycle. Unlike its 'fast-killing' variant, MRC-4 made only a transient appearance in the regional nodes, and failed to appear in the spleen. After intravenous injection MRC-4 was present in low titre in the peripheral nodes and spleen on D 2 but not thereafter, again contrasting with MRC-4 *f*, which increased in the nodes up to 3 weeks after injection, and attained high titres in the spleen between D 4 and D 11. Although the effect of a smaller dose of infective elementary bodies cannot be altogether discounted, these differences in ability to multiply correspond closely with those in guinea-pigs, in which the doses of MRC-4 and MRC-4 *f* were more nearly comparable (Blyth, 1967*b*).

These findings may be related to immunogenicity; whereas single subcutaneous or intravenous injections of MRC-4 *f* induced good immunity to conjunctival challenge, its parent strain failed in this respect; nevertheless, we have previously shown that repeated injections of MRC-4 confer a high degree of immunity (Collier, 1961; Collier & Blyth, 1966).

Our results lend weight to the supposition that the immunogenicity of TRIC

agents depends greatly on the attainment of high dosage. Although such doses can be attained by use of a strain that multiplies within the recipient, or by repeated injections, it might be more satisfactory to look to the development of highly concentrated inactivated vaccines for use in man.

SUMMARY

After a single subcutaneous injection into baboons the MRC-4 strain of trachoma/inclusion conjunctivitis (TRIC) agent underwent limited multiplication at the injection site, but was then eliminated rapidly from the skin and regional lymph nodes. Forty-eight hours after a single intravenous injection, but not thereafter, it appeared in the peripheral lymph nodes and spleen. The single parenteral injections failed to immunize baboons against conjunctival challenge with the homologous strain. These findings contrasted with those previously reported for the more virulent mutant, MRC-4 *f*, which multiplied readily in the skin, lymph nodes and spleen, persisted in these tissues up to 3 weeks after injection, and conferred good immunity to conjunctival challenge with MRC-4. The difference in behaviour of MRC-4 and MRC-4 *f* might be accounted for, at least in part, by the use of a smaller inoculum of live MRC-4; but similar findings in guinea-pigs, reported elsewhere, suggest that the differences observed are real. In conjunction with previous work, the present study suggests that the immunogenicity of TRIC agent is closely related to the mass of antigen that can be administered to or propagated within the recipient.

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