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Serotypes of trachoma agent isolated in The Gambia: with an observation on the relation between serotype and morphology

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SUMMARY

Of 60 TRIC agents isolated from Gambian children with trachoma, 25 were serotype 1 and the remainder type 2. There was a pronounced difference in the proportions of these types in the two villages studied. In the village with a predominance of type 2 strains, TRIC agents remained confined to 2 adjacent compounds over a 14 month observation period. All 19 type 1 strains examined were characterized by the appearance in yolk sac smears of compact aggregates of elementary bodies; such aggregates were seen in only 2 of 35 type 2 strains, and may reflect a chemical difference in the surface of the elementary bodies or in a substance elaborated during their replication.

INTRODUCTION

The *Chlamydia* causing trachoma and inclusion conjunctivitis (TRIC agents) were first grouped into serotypes by the mouse toxicity prevention test (Bell, Snyder & Murray, 1959; Bell & Theobald, 1962; Chang, Wang & Grayston, 1962). This method was later superseded by indirect immunofluorescence techniques that gave results corresponding closely with those of the mouse test, and by means of which the same serotypes can be elicited. In general, patients with ophthalmic TRIC infection where trachoma is endemic yield types 1, 1b and 2 (McComb & Bell, 1967) which correspond respectively to types A, C and B (Alexander, Wang & Grayston, 1967). By contrast, types D, E and F of Alexander and his colleagues are usually isolated from patients with TRIC agent syndromes other than typical ophthalmic trachoma, such as inclusion conjunctivitis or genital tract infections.

The distribution of serotypes isolated from trachoma varies from country to country. Wang & Grayston (1971) commented that type B (2) strains have been isolated in all endemic areas, whereas types A (1) and the related C (1b) strains are not found in the same area; the exception is Saudi Arabia, where the incidences of types 1, 1b and 2 in 338 strains were 37 %, 25 % and 38 % respectively (Nichols,

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von Fritzinger & McComb, 1971). It was of interest to determine what serotypes are prevalent in The Gambia, whether the distribution varies in different places within the country, and whether these findings would be of use in epidemiological studies. This study formed part of a larger longitudinal investigation on trachoma in Gambian children, other aspects of which have been previously reported (Sowa, Collier & Sowa, 1971; Collier, Sowa & Sowa, 1972).

MATERIALS AND METHODS

Provenance of strains

The TRIC agents were isolated from the eyes of trachoma patients, nearly all children, in two villages: Salekini, the large village on the north bank of the River Gambia, in which our other studies were undertaken; and Berending, a small isolated village south of the river and about 100 km. to the west of Salekini. They were collected during 6 surveys made in the period 1969–70.

Isolation of TRIC agents

TRIC agents were isolated from conjunctival scrapings by inoculating the yolk sacs of 6-day chick embryos (Sowa, Sowa, Collier & Blyth, 1965). The precautions taken to avoid cross-contamination included a lapse of at least 1 hr. between the harvest of yolk sacs from different batches, during which the working area was thoroughly swabbed with alcohol. Furthermore, almost none of the isolates were passaged more than twice before they were serotyped. From heavily infected yolk sacs 50 % crude suspensions in phosphate-buffered saline (Dulbecco & Vogt, 1954) were made and stored at −60° C. until use, when they were thawed and diluted to a 5 % concentration for making slide antigens.

Reference antigens

Dr Roger Nichols (Harvard School of Public Health) kindly provided the following strains in the form of 50 % crude yolk sac suspensions:

TRIC/1/ET/HAR-13/OT; TRIC/1b/SAU/HAR-32/OT;
TRIC/2/SAU/HAR-36/OT; TRIC/D/WAG/MRC-1/OT;
TRIC/E/USA-Cal-1/OT; TRIC/F/USA-Cal/Cal-9/ON.

The second element in the designation denotes the serotype (Gear, Gordon, Jones & Bell, 1963).

These suspensions were used as absorbing antigens and, diluted to 5 %, as slide antigens. The strains were used as received without further passage for all immunofluorescence tests done with donkey sera. For making the antigens used to immunize mice they were passaged once more in the chick embryo with stringent precautions to avoid cross-contamination.

The nomenclature of these serotypes has not yet been finally agreed; the workers at the Harvard School of Public Health designate the first three by numbers, whereas at the University of Washington, Seattle, letters are used for all serotypes. In this paper we shall use the Harvard system to designate serotypes 1, 1b and 2

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identified by McComb & Bell (1967), and referred to by the Seattle workers as A, C and B respectively. We shall, however, use the Seattle system to refer to types D, E and F that were first described by Alexander *et al.* (1967).

Typing sera prepared in donkeys

Antisera to the various serotypes were kindly provided by Dr Roger Nichols, and were cross-absorbed in our laboratory according to the methods of Nichols & McComb (1964) and Nichols *et al.* (1971). After absorption they reacted specifically with their homologous antigens at the following dilutions: type 1 serum at 1/8; 1b at 1/4; 2 at 1/16; D at 1/60; E at 1/56; F at 1/96. When tested against prototype strains and a number of freshly isolated strains these sera gave results identical with those with control sera absorbed in Dr Nichols's laboratory. The anti-donkey fluorescein isothiocyanate (FITC) conjugate was also supplied by Dr Nichols and was used at a dilution of 1/14 determined by chessboard titration.

Preparation of antisera in mice

Immunizing antigens. Stored 50% yolk sac suspensions of the reference strains were thawed in a water bath at 37° C., diluted to a concentration of 5% with saline buffered with 0·01 M phosphate (PBS) (Fothergill, 1964) and homogenized for 1 min. at full speed in an M.S.E. blender. They were centrifuged at 800 ***g*** for 10 min. at 10° C.; the middle layer was diluted fivefold and stored in 5 ml. amounts at −60° C.

Antisera. These were prepared by a method based on that of Wang (1971); groups of 6 Swiss albino mice were injected intravenously with 0·5 ml. of antigen on days 0 and 4; they were bled on day 11 and the pooled sera were absorbed with normal yolk sac. The degree of cross-reactivity with heterologous antigens was higher than reported by Wang, and the sera were therefore absorbed in the same way as the donkey sera. After absorption, the mouse sera did not react even at low dilution with heterologous antigens and were used at the following final dilutions: types 1, 2 and F at 1/16; 1b, D and E at 1/8. The anti-mouse FITC conjugate was obtained from Flow Laboratories and was diluted 1/10 for use.

Indirect micro-immunofluorescence tests

The method was similar to that of Wang (1971).

Slide antigens. With a dip pen 'dots' of slide antigen were deposited on a slide aligned over a template; the dots were grouped so that one drop of serum dilution would cover 4 antigen spots. Each slide accommodated 48 antigen spots, including positive controls of known serotype and negative controls made from normal yolk sac suspension. After drying in air the slides were fixed in acetone for 10 min. at room temperature and stored at −60° C.

Staining method. Slide antigens were thawed and dried in a stream of air. Drops of typing sera, appropriately diluted in PBS, were applied to the antigen spots and the slides were incubated in a humid atmosphere at 37° C. for 30 min. They were then rinsed in PBS, immersed in fresh PBS for 10 min. and dried. The appropriate

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Table 1. *Serotypes of TRIC agents isolated in two Gambian villages*

Village	No. of strains	Serotype 1	Serotype 2
Salekini	13	11	2
Berending	47	14	33
Totals	60	25	35

Table 2. *Distribution of TRIC serotypes in Berending*

Serotypes isolated	No. of families	No. of children yielding isolates
Type 1 only	3*	5
Types 1 and 2	2	10
Type 2 only	10	21

* All in one household (compound).

conjugate was then applied and the incubation and washing procedures were repeated. After a final wash in 75 ml. PBS containing 0·3 ml. of 1 % Evans blue as counterstain (Nichols & McComb, 1964) the slides were dried and mounted in glycerol:PBS, 9:1.

Ultraviolet microscopy. This was done as previously described for yolk sac slide antigens (Sowa *et al.* 1971), except that serum titration endpoints were taken as the first doubling dilution giving a fluorescence intensity of 2 with the set of graded density filters (Collier, 1968).

RESULTS

Comparison of results with donkey and mouse typing sera

Strains isolated during the first 5 surveys were typed with donkey sera; these reagents were exhausted by the time of the final survey, and recourse was had to the mouse sera that had been prepared for this contingency. Repeat tests with mouse sera on six type 1 and seven type 2 strains previously identified with donkey sera gave identical results.

Serotypes isolated

Table 1 shows that only serotypes 1 and 2 were isolated in the villages under study, and that neither type 1b, nor types D, E and F were detected. The relative proportions of types 1 and 2 varied in the two villages; in Salekini, 85 % of 13 strains were type 1, but in Berending, 70 % of 47 strains were type 2. In Berending, where the family distribution was studied, all the 21 children in ten families yielded only type 2 TRIC agents; the three families yielding only type 1 all lived in the same compound which was directly adjacent to the compound housing the two families yielding both types 1 and 2. (Table 2).

Morphological peculiarities of type 1 strains

We have previously described two strains of TRIC agent (G16 and G17) that differ from others in producing in yolk sac smears compact aggregates of elementary bodies that cluster densely around one or more vacuoles containing a

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Table 3. *Association of elementary body aggregates (EBA) with serotype*

Provenance of strains	EBA present (+) or absent (−)	No. of strains Type 1	Type 2	Totals
Salekini	+	10	2	12
	−	0	0	0
Berending	+	9	0	9
	−	0	33	33

carbohydrate, probably glycogen (Collier, 1959; Sowa & Collier, 1960). It was later noticed that these elementary body aggregates (EBA) were formed by about 25% of strains isolated in The Gambia. They continued to appear in all subsequent chick embryo passages of these strains, and tended to be more numerous in chick embryos dying late (say 9–10 days) after inoculation. Table 3 shows the distribution by serotype of EBA in strains in which they were specifically looked for. There was a close correlation between the presence of these bodies and serotype, since of 21 strains producing them, 19 were type 1; they were found in only 2 of 35 type 2 strains.

DISCUSSION

This is the first report of the serotyping of a significant number of TRIC agents isolated from ophthalmic trachoma in The Gambia. The apparent absence of serotypes other than 1 and 2 from the villages under study is interesting, particularly since the first trachoma agent isolated in The Gambia proved to be type D (Wang & Grayston, 1971). This was surprising, because the strain in question (MRC-1/OT, formerly G1: Collier & Sowa, 1958) came from the eye of an 8-year-old girl with typical ophthalmic trachoma and is thus unlikely to have originated from a genital tract infection. The findings reported here suggest that the G1 strain is not typical of the generality of Gambian trachoma agents. The only other serotype detected by us was type F, isolated in Bathurst, the capital, from the eye of a newborn baby and from its father's urethra (Collier, Sowa & Sowa, 1969).

The finding of only 2 serological varieties of trachoma agent in the areas under study obviously limits the use of serotyping for researches on epidemiology in general and transmission in particular. It is however noteworthy that in Berending, despite the frequent movements of people between households, type 1 strains remained restricted to two adjacent compounds over a period of at least 14 months, suggesting that ophthalmic trachoma agents are not readily disseminated without close and frequent personal contact. Nichols and co-workers (1971) reported pronounced differences in the prevalence of types 1 and 2 in two Saudi Arabian villages only a few hundred yards apart and with considerable traffic between them; they considered that transmission of trachoma takes place largely within the family, and as far as they go our observations support this inference.

The close correlation between serotype and the appearance in yolk sac smears of dense aggregates of elementary bodies is particularly interesting. It must be stressed that these EBA are not merely local concentrations of free elementary

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bodies but discrete structures that maintain their identity in suspension and resist centrifugation, freezing and thawing. Strains that produce them seem to do so indefinitely; we have observed them after 50 yolk sac passages of strain MRC-17/OT (formerly G17), in which they were first noticed and which later proved to be type 1 (Alexander *et al.* 1967). They can be readily identified in sections of chick embryo yolk sac infected with this strain (Sowa & Collier, 1960) but one of us (L.H.C.) has also observed similar objects in sections of yolk sac infected with MRC-4/ON (formerly LB4) at its second passage in chick embryos after isolation. This strain came from the eye of an English baby with neonatal inclusion conjunctivitis and is type 2; by contrast with the type 1 strains, yolk sac smears – as opposed to sections – do not contain EBA, which therefore appear to disrupt much more easily than those of the type 1 strains; but our experience with sections is too limited to support generalizations in this direction. Nevertheless, our observations on these Gambian strains do suggest that type 1 TRIC agents differ from type 2 in terms of the chemistry of the elementary body surface or of some substance elaborated during replication. It remains to be determined whether the binding material or the contents of the central vacuole – which seems to contain much carbohydrate – are endowed with serological specificity; if so, the EBA might provide a good source of type 1 antigen.

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