Antichlamydial antibody in tears and sera, and serotypes of *Chlamydia trachomatis* isolated from schoolchildren in Southern Tunisia

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SUMMARY   A predominance of TRIC serotype A has been isolated from schoolchildren in a population in Southern Tunisia with severe hyperendemic trachoma. The serotyping results correspond precisely with the serological findings in patients' tears and sera. Geometric mean titres of serum or tear antibody in defined populations or areas can thus give a useful indication of the prevalent serotypes.

Collection of tear fluids on sponges is a more practical method than collection by filter paper strips and gives higher levels of antibody.

The presence of antibody to *Chlamydia trachomatis* in tears correlates well with the presence of infectious agent in the eye and with the intensity of conjunctival inflammatory disease. The measurement of antichlamydial tear antibody can thus provide a meaningful index of the prevalence and intensity of active trachoma in a population. The role these antibodies may play in resistance to re-infection is not yet clear.

The development of the microimmunofluorescence serological typing test for *Chlamydia trachomatis* (Wang and Grayston, 1970; Treharne *et al.*, 1971) has greatly facilitated the study of the TRIC agents responsible for hyperendemic trachoma of eye-to-eye transmission, and the TRIC agents of paratrachoma (both ocular and genital) of sexual transmission, with occasional transfer to the eye (Jones, 1975; Treharne *et al.*, 1977).

The microimmunofluorescence method has also been applied to the measurement of type-specific antichlamydial antibody in serum (Dwyer *et al.*, 1972; Hanna *et al.*, 1972; Jones and Treharne, 1974; Philip *et al.*, 1974; Wang and Grayston, 1974) and in tears (McComb and Nichols, 1970; Hanna *et al.*, 1973; Briones *et al.*, 1975).

The present study of schoolchildren with active hyperendemic trachoma in southern Tunisia presents the results of type-specific antibody measurements in tears and sera, and correlates these with the isolation of *C. trachomatis* in cell culture and with

the serotyping of these isolates. The correlation of these results with the intensity of conjunctival inflammatory disease is presented. Alternative methods of collection of tears for antibody measurements are evaluated, and the reliability and repeatability of the serological data are discussed.

Materials and methods

POPULATION STUDIED
Children in the first class in the school at Douz, southern Tunisia, were examined in November 1970. Physical signs of trachoma were scored, using a Haag-Streit 900 slit lamp in a long-term study of the natural history and the control of trachoma (Dawson *et al.*, 1974; Dawson *et al.*, 1976). The method of scoring signs used has been published elsewhere (Dawson *et al.*, 1975).

CONJUNCTIVAL SPECIMENS
Methods of ocular examination, assessment of the intensity of conjunctival inflammatory disease in trachoma, and methods of collection of specimens for the isolation of *C. trachomatis* are reported

elsewhere (Darougar *et al.*, 1971a; Darougar and Jones, 1971; Darougar *et al.*, 1971b; Dawson *et al.*, 1975).

SEROTYPING

Isolates from cell culture were inoculated into yolk sacs of developing chick embryos to produce antigen for use in the micro-IF test (Treharne *et al.*, 1973). Methods used in the micro-IF test have been described previously (Treharne *et al.*, 1971; Treharne *et al.*, 1972).

SERA

Blood was taken by venepuncture. All sera were kept chilled during transport and were stored at −20°C in the laboratory prior to titration.

TEARS

Fluid was collected from the conjunctival sac by different methods for each patient.

Filter paper. Sterile filter paper strips (Whatman No. 41) approximately 40×6 mm were used. The strip was bent one-third along its length and the short portion inserted into the left lower fornix. It was allowed to remain there until the strip was completely saturated, after which it was removed to a tightly-stoppered plastic capsule containing 0·2 ml of 0·01 M phosphate buffered saline (pH 7·2). Capsules were labelled and placed in a liquid nitrogen refrigerator for transport to the laboratory in London.

Cellulose sponge. A dry, sterile cellulose sponge (Spontex Ltd., Croydon, England; C-pore grade) approximately $5 \times 2 \times 1$ mm was placed in the right lower fornix. When the sponge was completely saturated it was stored in a tightly-stoppered plastic capsule containing 0·2 ml of 0·01 M phosphate buffered saline at pH 7·2 and transported to the laboratory in a liquid nitrogen refrigerator.

DETECTION OF ANTIBODY

All sera were absorbed before titration for 60 minutes at 37°C and then at 4°C overnight, with equal volumes of 40% (w/v) normal yolk sac. After absorption sera were titrated immediately, starting at a dilution of 1 in 8. As the volume of tears was so small, they were not absorbed. Filter paper strip collections and sponge collections were titrated separately at a starting dilution of approximately 1 in 4.

The method of detection of type-specific antibody has been described previously (Dwyer *et al.*, 1972). In this study all sera and tears were titrated against 9 different TRIC agent serotypes (*A, B, C, D, E, F, G, H,* and *I*), 3 lymphogranuloma venereum (LGV) serotype antigens (L1, L2, and L3), and 1 representative antigen from *Chlamydia* subgroup B *Chlamydia psittaci* (LGV (JH) strain).

Serum and tear titrations were carried out with a fluorescein isothiocyanate conjugated antihuman globulin of swine origin (Nordic Ltd.), with the addition of a 1:40 dilution of rhodamine conjugated bovine albumin used as a counterstain.

Results

TRIC AGENT SEROTYPES ISOLATED

Twenty-two out of the 23 isolates obtained were serotyped. Sixteen of these were TRIC type A, 5 TRIC type B, and 1 TRIC type C.

TYPE-SPECIFIC ANTIBODY IN SERA

Sera were collected from 94 and tears from 71 out of a total of 94 schoolchildren, between the ages of 6 and 10 years, examined in this study. Fig. 1 shows the geometric mean titre of the sera from 70 patients whose titres were 1 in 8 or greater. The highest geometric mean titre was to TRIC type A, which correlates well with the finding that this was
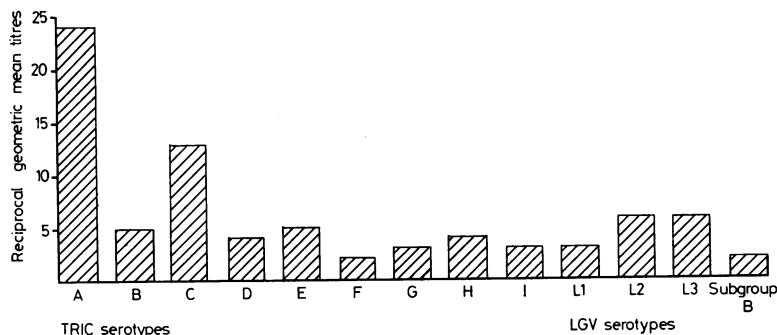

Fig. 1 *Geometric mean titres of type-specific antibody to chlamydial serotypes in 70 sera from children with trachoma in southern Tunisia*

TRIC type A

50
40
30
20
10

Sera ■
Tears □

Reciprocal geometric mean titre

TRIC type B

10
5

A   B   C   D   E   F   G   H   I   L1   L2   L3   Subgroup
TRIC   serotypes   LGV serotypes   B
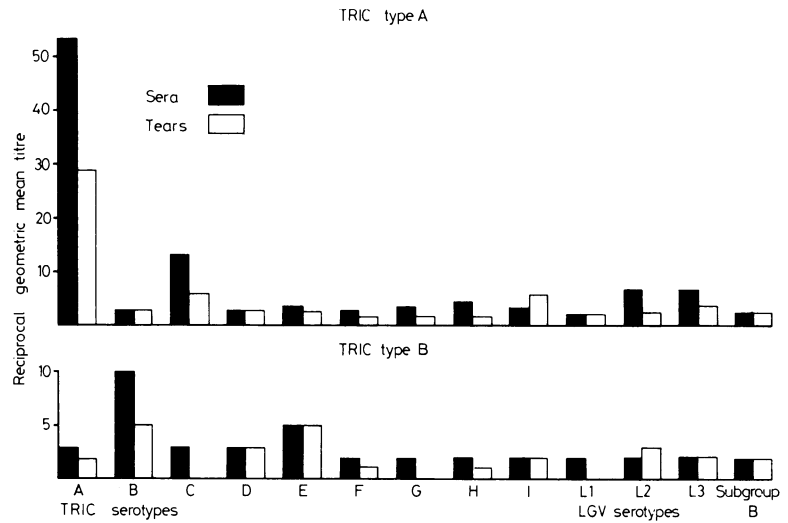
Fig. 2   *Geometric mean titres of type-specific antibody in sera and tears in 16 children whose isolates were serotyped as TRIC type A and 5 children whose isolates were serotyped as TRIC type B*

the predominant serotype isolated in this area. When the serum geometric mean titres were measured from those 16 patients whose isolates were serotyped as TRIC type A, a much sharper definition of the type-specific antibody profile appeared (Fig. 2). In each case the known cross-reaction between serotypes A and C was apparent.

Similarly, Fig. 2 shows the geometric mean titres of type-specific antibody levels in 5 children whose isolates typed as TRIC type B.

Examination of the distribution of serum antibody titres in isolation-positive and isolation-negative patients showed (Table 1) that 13 out of 21 isolation-positive patients (62%) had titres of 1 in 64 or greater, whereas only 10 out of 73 isolation-negative patients (14%) had this level of serum antibody. The geometric mean titre was 1 in 39 for isolation-positive patients and 1 in 9 for isolation-negative patients. Within those that were isolation-positive there was no relationship between the level of antibody in sera or tear fluids and the amount of viable TRIC agent present in the conjunctiva, as estimated by the number of inclusions detected in the first passage cell culture.

TYPE-SPECIFIC ANTIBODY IN TEARS
A similar distribution was demonstrated for tear antibody levels except that many fewer (20%) isolation-negative patients had antibody levels of 1 in 8 or greater (Table 2). Table 3 compares the distribution of antibody levels collected either by sponges or by filter paper strips. The percentage of positive titres was slightly higher in those specimens collected by sponge, though the difference between the 2 methods of collection did not attain statistical significance. But the difference in the geometric mean titres, i.e., 1 in 21 for sponges and 1 in 15 for filter papers was significant (P = 0·01).

The correlation of titres in matched sera and tear specimens showed wide variation. Of the children studied 81% had antibody in their sera at 1:8 or greater titre, while only 42% had antibody at this titre in their tears (Table 4). However, antibodies were rarely found in the tears unless they were also present in the serum.

RELATION OF TYPE-SPECIFIC ANTIBODY TO SEROTYPES
The pattern of antibody to chlamydial serotypes in

Table 1   *Distribution of antibody titres* in sera of Tunisian schoolchildren*

Category	No. of patients	Reciprocal titres*								Geometric mean titre†
		<8	8	16	32	64	128	256	512	
Isolate +ve	21	2			6	10	3		—	39·0
Isolate –ve	73	22	11	11	19	6	4		—	9·0

*Highest reciprocal titres.   †Titres 1 in 8 or greater

Table 2 *Distribution of antibody titres* in tears of Tunisian schoolchildren*

Category	No. of patients	Reciprocal titres*							Geometric mean titre†
		<8	8	16	32	64	128	256	
Isolate +ve	20	5	4	2	5	2	1	1	13·0
Isolate −ve	51	41	6	2	1	1	—	—	2·0

*Highest reciprocal titres. †Titres 1 in 8 or greater

Table 3 *Distribution of tear antibody titres in 71 matched specimens collected by sponge and filter paper*

Collection	Reciprocal titres							Per cent positive*	Geometric mean titre of positives*
	<8	8	16	32	64	128	256		
Sponge	51	8	2	5	4	1	—	28	21·0
Filter paper	56	9	3	1	1	—	1	21	14·6

*Titre of 1 in 8 or greater

sera and tears of 6 patients is shown in Table 5. In no case was the antibody pattern monospecific for a serotype different from that isolated. In 14 out of the 16 patients whose isolates were serotyped as TRIC type A the highest serum or tear antibody level was directed against the type A serotype antigen. Of the remaining 2 patients 1 had no antichlamydial antibody titre, and no serum or tears were collected from the other. Four out of 5

patients whose isolates were serotyped as type B gave highest levels of serum or tear antibody against the type B serotype antigen; no antichlamydial antibody could be detected in the other patient. In the single patient whose isolated serotyped as TRIC type C no antichlamydial antibody could be detected in either serum or tears. Although antibody titres in sera generally exceed those found in tears, the profile of cross-reactivity to the various

Table 4 *Relationship of antibody prevalence in matched sera and tears*

Total no. of matched sera and tears	Absence of antibody in both serum and tears		Presence of antibody in serum only*		Presence of antibody in tears only*		Presence of antibody in both serum and tears*	
	No.	%	No.	%	No.	%	No.	%
67	11	16	28	42	2	3	26	39

*Presence of antibody in sera and tears 1 in 8 or greater

Table 5 *Pattern of antibody activity to chlamydial serotypes in sera and tears of 6 isolate-positive patients*

Patient no.	Isolate serotype	Sera/ tears	Reciprocal titres													Subgroup B
			A	B	C	D	E	F	G	H	I	L1	L2	L3	
160	A	S	128	.	16	.	.	.	.	.	.	.	.	.	8
		T	32	.	.	.	.	.	.	.	.	.	.	.	.
273	A	S	128	8	64	8	8	16	16	16	8	16	32	.	8
		T	256	8	64	8	.	8	.	.	16	8	4	.	16
338	A	S	64	.	8	.	16	.	.	.	.	.	8	8	.
		T	8	.	.	.	.	.	.	.	.	.	.	.	.
371	A	S	64	.	32	.	.	.	.	.	.	.	8	.	.
		T	128	.	8	.	.	.	.	.	.	.	4	.	8
219	B	S	.	32	.	8	16	.	.	.	.	.	.	.	.
		T	.	.	.	.	.	.	.	.	.	.	.	.	.
324	B	S	.	128	.	8	16	.	.	.	.	.	16	.	.
		T	16	64	16	32	64	.	.	.	.	.	64	.	4

chlamydial serotypes in any individual patient was usually similar for both serum and tears.

INTENSITY OF CONJUNCTIVAL INFLAMMATION AND ANTIBODY

Table 6 shows the distribution of antibody titres in sera and tears in relation to the intensity of conjunctival inflammatory disease (Dawson *et al.,* 1975). There is good correlation of high antibody levels in the tears with severe inflammatory disease in trachoma, the antibody levels diminishing as the disease becomes less active. Antibody levels in sera, however, can be detected at fairly high levels even in the mild stage of the disease. Geometric mean titres of sera and tears shown in Table 6 reflect the previous findings that antibody levels in sera tend to remain at a high level throughout the severe, moderate, and mild clinical grades of inflammatory disease, whereas antibody levels in tears are at high levels only in the more severe grades of inflammatory disease.

MACCALLAN STAGES OF TRACHOMA AND ANTIBODY

There was little correlation between the MacCallan stages of trachoma and the presence of antibody in either serum or tears.

Discussion

Using experimentally infected monkeys Wang and Grayston (1971) demonstrated an antibody response in sera and tears that was strain-specific to the infecting TRIC serotype. McComb and Nichols (1970) showed by a complex cross-absorption technique that antibody in eye secretions faithfully reflected the type of TRIC agent infecting the eye.

Studies in our laboratory (Darougar *et al.,* 1971b; Treharne *et al.,* 1971; Treharne *et al.,* 1972; Jones,

1974) have shown, in agreement with the work of others (Wang and Grayston, 1970; Wang and Grayston, 1974), that TRIC agent serotypes A, B, and C are mainly responsible for ocular infection in hyperendemic trachoma areas.

In the present study the estimation of geometric mean serum and tear titres to TRIC agent serotypes in a village with hyperendemic trachoma in southern Tunisia gave a clear indication of the prevalent infecting TRIC serotype in that area, namely, type A.

In the same population of Tunisian children other workers (Hanna *et al.,* 1972) found that nearly 70% of all TRIC serum antibody was directed against types A or A C. This compares well with our findings and demonstrates the repeatability of results between 2 laboratories. Both findings are now substantiated by the fact that 76% of the serotypes we isolated (17 out of 22) were of the A or C types.

In every case where the infecting TRIC agent was serotyped the antibody level in both serum and tear fluids was highest to the homologous serotype. Thus the geometric mean antibody levels in defined populations or areas would appear to be a useful guide to the predominant TRIC serotype(s) present.

Two methods of collection of tear secretions were used in this study. The filter paper strips when fully saturated contained 0·04 ml of tear fluid, whereas the sponges when saturated contained 0·1 ml of tear fluid. Collection of tears using cellulose sponges was easier, quicker, and induced less lacrimation than collection by the filter paper. Antibody titres obtained by collection with sterile sponge inserts were significantly higher than those titres obtained with filter paper strips (P=0·01). In addition elution techniques were far simpler with the sponges. For these reasons we recommend them as the preferred method of tear collection.

The levels of antibody in both serum and tear

Table 6 *Clinical activity of disease correlated with distribution of antibody levels, geometric mean antibody titres, and percentage of sera and tears positive*

Clinical activity	Sera/ Tears	Reciprocal titres							Geometric mean titre*	Per cent positive*
		<8	8	16	32	64	128	256		
Severe	S	2	1	1	1	3	3		51	82
	T	3	1		3	2		1	43	70
Moderate	S	3	1	5	9	4	4		37	88
	T	18	3	2	2	1		—	12	31
Mild	S	6	5	3	12	5			25	81
	T	25	4	—			1		7†	17

*Positive = 1 in 8 or greater.   †Including 6 patients with a titre of 1 in 4

fluid correlated positively with isolation of the agent from the conjunctiva, the highest antibody levels being found in those patients who were isolation-positive.

In all but 2 instances antibody levels in sera were as high as, or higher than, the levels in matched tears, which is in agreement with the findings of Hanna et al. (1973) and suggests that most of the antibody in tears is due to transudation from the serum. Higher tear antibody levels were found in those patients with the greatest intensity of conjunctival inflammation, in whom the conjunctival vessels might reasonably have the greatest increase in conjunctival capillary permeability. However, in this study as in our earlier work a small proportion of persons had higher levels of antibody in their tears than in their sera (Darougar et al., 1971a). Thus the possibility of local production of antibody in the conjunctiva is not precluded.

The prevalence of antibody in tears parallels the amount of TRIC agent in the conjunctiva as detected by immunofluorescent staining of inclusions in conjunctival scrapings (Hanna et al., 1973). We used the irradiated McCoy cell method for isolating Chlamydia trachomatis from the conjunctiva. This has been shown to be more sensitive than the immunofluorescent staining technique (Jones, 1974) and gave a positive correlation between the presence of viable agent and a tear antibody level of 1 in 8 or greater. However, within those cases that were isolation-positive we were unable to find any correlation between the level of tear antibody and the amount of viable agent in the conjunctiva.

In this study an antihuman globulin conjugate was used to detect antibody to C. trachomatis in both sera and tears. Results from our laboratory using a specific antihuman IgM FITC conjugate suggest that the presence of antichlamydial IgM in patients' sera correlates well with active TRIC agent infection (Treharne et al., 1977). Furthermore, work by others (Murray et al., 1973) on experimental conjunctivitis in the guinea-pig would suggest that the most important immunoglobulin in tears is secretory IgA (SIgA). This antibody appears to be associated with resistance to re-infection in this model and is not normally found in serum. Detection of SIgA antibodies directed against TRIC agent in tears using the appropriate anti-SIgA fluorescent conjugate may well provide useful information on the local production of highly specific TRIC agent antibodies in tears.

High levels of demonstrable serum antibody appear to persist in the absence of isolatable C. trachomatis in the conjunctiva and when the clinical activity of the disease is mild. The presence of tear antibody, on the other hand, appears to be closely related to the presence of agent in the conjunctiva and the intensity of inflammation. The measurement of tear antibody should therefore provide a meaningful type-specific index of current ocular infection due to various serotypes of C. trachomatis. The measurement of these tear antibodies should also provide a reliable index of both the prevalence of active trachoma and the intensity of inflammation, which correlates with the public health importance of the disease. The simultaneous measurement of serum antibodies would provide a further index of the prevalence of healed trachoma and cases with mild degrees of inflammatory disease in a population under study.

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