

Complement-fixation and complement-fixation-inhibition titres against the *Bedsonia* antigen* in sera of trachoma patients

BY A. L. TERZIN AND B. V. BIRTAŠEVIĆ

Medical Faculty and HE Military Laboratory, Sarajevo, Yugoslavia

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INTRODUCTION

Since the widespread adoption of T'ang's procedure (T'ang, Chang, Huang & Wang, 1957) for the isolation and propagation of the trachoma virus in chick embryo, some of the previously conflicting claims regarding serological problems connected with trachoma have been elucidated. At the same time some new questions have been raised.

It seems to be now established (Murray, Bell, Hanna, Nichol & Snyder, 1960; Grayston, Wang, Woolridge, Yang & Johnston, 1960) that trachoma strains share with the group of psittacosis-lymphogranuloma (LGV) viruses (the *Bedsonia* group) the common heat-stable antigen, as detected by titration of antigens prepared from various trachoma strains against known psittacosis or LGV-positive sera.

In accordance with Bedson's early findings (1933) on psittacosis, Grayston *et al.* (1960) reported that 'purified' trachoma-specific antigens react in complement-fixation (CF) tests with sera of trachoma patients. However, in contrast with what is known from earlier studies on psittacosis, they also reported that sera of trachoma patients did not react in CF-tests with group-specific, heat-stable *Bedsonia* antigens, even when tested against group-specific CF-antigens prepared from strains of trachoma virus.

We have been unable to find published information regarding the question as to whether trachoma patients develop group-specific antibodies detectable by CF-inhibition rather than by complement-fixation tests (Terzin, 1960). The primary purpose of the present investigation was to test sera of trachomatous persons for the presence of group-specific CF-inhibiting antibodies. Owing to some unexpected details revealed during the investigation this report also presents some findings apparently unrelated to the primary purpose of the study.

In order to complete the data presented here, a separate communication will be prepared to report on the results obtained by CF-tests performed on the same serum samples, with specific trachoma antigens, presently under investigation.

* The term '*Bedsonia* antigen' is used here to indicate the *group-specific* heat-stable antigen of the psittacosis-LGV group of viruses (Terzin, 1960).

MATERIALS AND METHODS

Patients' sera

The sera used in these studies were collected from 168 cases of trachoma diagnosed and selected by ophthalmologists, some time ago. All patients had been stationed in the Working Collective T, as cases found to be fit for field work in forestry. All patients belonged to the age group 20–27 years, the great majority being of the age of 21 years. About 97% of the patients tested came from areas of Yugoslavia known to be endemic for trachoma; 44% of the cases belonged to families with two or more trachoma cases recorded in each.

In the periods prior to their being stationed in 'Collective T', the majority of the patients had been treated in various ways, for periods of a few months to several years, but only a few of them properly. Several months prior to bleeding, all were, however, treated topically with aureomycin.

Classification of trachoma cases

One to three days prior to the bleeding, each of the 168 patients had been examined at the field clinic, mostly by inspection of the everted eye-lids only. They were classified according to the criteria proposed by MacCallan (1936) (see also, Expert Committee on Trachoma, 1952). No patient was found to reveal symptoms characteristic for stage I of trachoma; 132 of the patients were recorded as being in stages II, III or IV. The remaining 36 cases were doubtful either as to the stage of the disease, or as to being clinical trachoma at all. When analysing the results reached, we shall deal only with those 132 cases of trachoma which had been grouped according to stages of the disease. Some of the cases grouped in stages II or III were relapses. From the majority of the patients it was not possible to obtain reliable data which would be necessary for a proper record of all relapses.

Cases grouped in stage II showed scanty secretion or none, follicles and/or papillary hypertrophy at the upper tarsus, with or without ulcerations. Some had epithelial keratitis or pannus and none of them showed signs of scarring.

In stage III were grouped cases showing cicatrization, with infiltration and follicles in regression, with or without ulcerations. Some cases showed deformations of the eye-lids, others revealed opacities of the cornea, or signs of epithelial keratitis or pannus.

In stage IV were grouped cases showing definite residual lesions such as signs of conjunctival atrophy or cicatrization. The stage of 'healed trachoma' was manifested in all cases by an absence of signs of inflammation or infiltration.

Serology

A total of 168 serum samples were tested by CF and CF-inhibition tests. Both the technique and the reagents used in performing these tests were those described previously (Terzin, 1960). All sera were titrated in twofold serial dilutions, from 1/2 to 1/128. In the CF-test each serum was titrated versus two dilutions of the antigen, one containing four and the other 16 units of the ether-soluble *Bedsonia*-antigen prepared by the procedure described elsewhere (Terzin, Matuka,

Fornazarić & Hlača, 1961). In CF-inhibition tests, dilutions of sera were titrated against an indicator system containing two units of the same antigen, and two units of a psittacosis-positive human serum. None of the 168 specimens tested had been found anti-complementary, nor did any react with the control antigen prepared from normal yolk membranes.

Statistical computations

The χ^2 tests for measuring the significance in proportions, the *t*-test values for estimation of the significance of difference between two means, and the correlation coefficients and their significance for estimating degrees of association, have been calculated, each according to two or more of the procedures described by Chambers (1952) and/or by Bancroft (1959). In the computations performed, all titres of less than two had been taken as 1/1.

Table 1 presents all the data from which any of the calculations referred to in the present report can be computed.

RESULTS AND CONCLUSIONS

The CF-inhibition tests

All 132 serum samples of confirmed clinical trachoma cases, as well as the sera of the 36 doubtful cases, revealed negative results when tested by the CF-inhibition test.

Controls were set up daily with sera of known positive CF-inhibition titres, to check the sensitivity of the indicator system used, as well as other controls to check the amounts of the various reagents used in the test.

On the basis of the well-controlled negative results obtained with sera of 168 patients, which included 132 specimens from recognized cases of stages II, III or IV of the disease, we concluded that sera of individuals with clinical trachoma do not give a positive CF-inhibition test, when titrated against an indicator system consisting of Bedsonia antigen and anti-Bedsonia serum. In other words, trachoma patients do not develop group-specific CF-inhibiting serum-antibodies.

The CF-tests

Although all 168 serum samples had been tested by CF-test versus the group-specific Bedsonia antigen, only the results of those 132 specimens whose donors we were able to group in one of the recognized stages of clinical trachoma will be considered.

Table 1 presents the number of the sera tested, the number of positives found, the height of the titres observed, the duration of the disease, as well as the clinical stage of trachoma to which the respective patients belong.

As shown in Table 2 the incidence of trachoma cases with a duration of 8 years or longer was significantly higher both in groups III and IV, than in the group II. The χ^2 values obtained being 7 and 10 respectively, both values indicating with a probability less than 0.01 that the differences in the incidences observed could have occurred by chance. On the other hand, the incidences of trachoma cases with a duration of 8 years or longer appearing in groups III and IV reveal no

significant differences, the χ^2 value found being less than 0.2, indicating with a probability of more than 0.5 that the difference observed could have occurred by mere chance. In other words, the group of stage II trachomas comprises significantly more cases with duration of illness below 8 years, than the groups of stage III or stage IV trachomas do. The last two groups showed no significant difference in respect of the duration of the disease.

Table 1. *CF-tests with Bedsonia antigen. Data on 132 trachoma cases*

Stage	Titre	No.	Per-centage	Duration (years)															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
II	< 2	28	65.0	6	3	3	4	4	2	1	—	1	1	1	—	2	—	—	—
	2	7	16.3	3	2	1	—	—	—	1	—	—	—	—	—	—	—	—	—
	4	5	11.6	1	2	1	—	—	1	—	—	—	—	—	—	—	—	—	—
	8	3	6.9	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—
	All	43	99.8	10	8	7	4	4	3	2	—	1	1	1	—	2	—	—	—
III	< 2	31	75.5	—	4	5	3	2	3	2	2	—	6	2	1	1	—	—	—
	2	5	12.2	—	1	—	—	—	—	—	1	1	—	1	—	1	—	—	—
	4	1	2.4	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
	8	3	7.3	—	1	—	—	1	1	—	—	—	—	—	—	—	—	—	—
	16	1	2.4	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
All	41	99.8	—	6	5	5	3	4	2	3	1	6	3	1	2	—	—	—	
IV	< 2	29	60.5	—	2	4	3	3	5	2	1	2	1	1	3	—	2	—	—
	2	6	12.5	—	—	—	1	—	1	—	1	1	—	—	—	—	1	—	1
	4	6	12.5	—	—	—	1	1	—	—	2	1	—	—	—	1	—	—	—
	8	6	12.5	—	—	—	1	1	1	1	—	—	1	1	—	—	—	—	—
	64	1	2.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
All	48	100.1	—	2	4	6	5	7	3	4	4	2	2	3	1	3	1	1	

Table 2. *Analysis of the incidence of cases with duration longer than 7 years in various stages of our trachoma cases*

Stages	Duration (years)	Mean duration (years)	No. of cases with duration (years)		χ^2 values comparing the respective group of data with other groups	
			1-7	8-16		
II	1-13	3.977	38	5	7:III	10:IV
III	2-13	6.440	25	16	7:II	< 0.2:IV
IV	2-16	7.500	27	21	10:II	< 0.2:III
All	1-16	6.023	90	42	—	—

As seen from the data presented in Table 1, regardless of whether titres of 1/2, 1/4 or 1/8 were taken as critical, the highest percentage incidence of positives, as well as the highest absolute titre (1/64) observed, occurred in the group of stage IV cases. When analysed statistically, these apparent differences were found to be not significant. However, as shown in Table 3, the mean titres revealed by the group of stage IV cases were significantly higher than those revealed either by the group of stage II, or of stage III trachomas. This was true also when the computations were made without the sample showing a titre of 1/64, which belonged to the group of stage IV cases. The probability levels presented in Table 3 were

taken from the table of Fisher and Yates, corresponding to the respective degrees of freedom and corresponding to the respective *t*-values obtained for the pairs of the groups compared (see Bancroft, 1959). The groups of variables compared were the titres found in stages II, III and IV, with the sample showing the titre 1/64, and in stage IV without the sample showing the titre 1/64.

Table 3. *Analysis of the height of titres revealed by trachoma cases of various stages*

Stages	Titres revealed		No. of cases	Probability levels of <i>t</i> -values comparing the respective group of data with other groups	
	Maximal	Mean			
II	1/8	1/2.000	43	> 0.05:III	< 0.001 } ^{IV} (< 0.001)
III	1/16	1/2.073	41	> 0.05:II	< 0.001 } ^{IV} (0.001)
IV	1/64 (1/8)	1/3.688 (1/2.404)	48 (47)	< 0.001 } ^{II} (< 0.001)	< 0.001 } ^{III} (0.001)

Values in parentheses refer to 47 samples belonging to stage IV, without the sample showing a titre of 1/64.

Table 4. *Measurement of association between duration of the disease and CF-titres revealed*

Stages	No. of pairs of variables	Correlation coefficients	Levels of significance
II	43	-0.215	$P > 0.1$
III	41	-0.189	$P > 0.1$
IV	48 (47)	+0.296 (+0.037)	0.02 < P < 0.05 ($P > 0.1$)

Values in parentheses refer to 47 samples belonging to stage IV, without the sample showing a titre of 1/64.

It was shown in this way that although stages III and IV showed no significant differences in respect to the duration of the disease, significant differences were revealed when the mean titres of these two groups were compared. These findings suggest that the significantly higher mean titres revealed by the stage IV group (as compared with significantly lower mean titres found in stages II or III) have been induced by factors other than the duration of the disease. This assumption seems to be confirmed also by the correlation coefficients presented in Table 4. The data presented show that in the stage II and III groups, no correlation exists between the duration of the disease and the height of the CF-titres revealed. In the group of stage IV cases, if any relationship exists between the duration of illness and the titre of CF-antibodies, it was a positive correlation of a very low degree.

As seen from Table 1 the incidence of positive reactors does not vary significantly among the three groups of trachoma patients, the percentage incidence of Bedsonia-positive reactors found being comparable with that found in other groups of apparently healthy Yugoslav population not affected by trachoma (Terzin,

Fornazarić & Hlača, 1957). Adding to this the fact that, at least in stages II and III, and probably also in stage IV, no correlation exists between the anti-Bedsonia CF-titres found and the duration of the disease, it seems reasonable to conclude:

(a) The anti-Bedsonia CF-titres found in sera of trachoma patients represent residual titres developed in response to past infections with agents other than trachoma.

(b) In the group of stage IV trachomas of our material, the mean values of the anti-Bedsonia CF-titres were significantly higher than those found in the groups of stage II or III.

(c) The factors responsible for that difference found in mean titres must be other than the duration of the trachoma.

DISCUSSION

Our findings seem to confirm the observations made by Grayston *et al.* (1960) about the absence of group-specific CF-antibodies from the blood of trachoma patients. We may add to that, that sera of trachoma patients do not contain group-specific CF-inhibiting antibodies either. No hypothesis is advanced to explain these observations.

With regard to the incidence of positive reactors, as well as the height of the titres observed, according to Grayston *et al.* (1960), the second stage of the disease was the most active, if the sera were tested with purified, specific CF-antigen. In contrast, in our experience with the group-specific antigen, sera of patients of stage IV trachoma were found to be the most reactive, as revealed by a suggestive but not significantly higher incidence of positives in the group, by the highest peak titre of 1/64 shown by one sample occurring in that group, and by a significantly higher mean titre revealed by the group of stage IV as a whole.

Three hypotheses have been advanced to explain the appearance of significantly higher mean titres against the group-specific CF-antigen, as revealed by the group of stage IV trachoma cases. The explanation sought was one which would be consistent with the three conclusions (*a*, *b* and *c*) set out above, at the end of 'Results and Conclusions'.

One of the hypotheses supposes that the formation of group-specific antibodies in trachoma is restricted to stage IV of the disease. One may think of a local production in the affected tissue, and of a continuous penetration of the soluble antigen into the whole organism. It was supposed that the continuous 'saturation' of the organism with the soluble antigen might prevent the appearance of detectable group-specific antibodies, simulating thus a sort of immunological tolerance of the infected organism versus the soluble antigen, persisting as long as the 'secretion' of the soluble antigen, that is, until the end of stage III trachoma. Although apparently consistent with the three conclusions reached, this hypothesis is contradicted by several other arguments, the last being the crucial one, for it implies a significantly greater incidence of Bedsonia-positive reactors in the group of stage IV trachoma than in stages II or III. Our findings showed that the differences in incidence, if any, are insufficient to support the hypothesis advanced.

The second hypothesis advances the view that the group-specific titres developed in response to past infections, with *Bedsonia* viruses other than trachoma can be enhanced by subsequent trachoma infections to ensure a significantly higher mean titre in the group of stage IV trachomas. By additional speculation one might supplement this hypothesis to make it more consistent with all three of the conclusions reached. Nevertheless, this hypothesis becomes untenable when confronted with some facts of which only one is mentioned here. It would be very hard to devise convincing reasons to explain why the enhancement of previously developed CF-titres is restricted to the group of stage IV trachoma cases. In fact, Collier, Duke-Elder & Jones (1958) brought forward suggestive evidence to show that residual CF-titres against the group-specific psittacosis antigen can be enhanced by subsequent trachoma infection. However, the increase of anti-*Bedsonia* titres observed by them was detected 3 and 6 weeks after the infection with trachoma virus, that is, much earlier than stage IV of the disease. The objection presented seems to be sufficient to invalidate the second hypothesis.

The last hypothesis offered here for discussion seems to be the least sensational and the most consistent with the facts observed so far.

It has been shown that no significant difference exists in respect of the duration of the disease, between our groups of stage III and stage IV trachomas. However, the mean titre revealed by the group of stage IV proved to be significantly higher than the mean titre revealed by stage III trachomas. Apparently some reactive individuals have been included in the stage IV cases, with enhanced capacities to maintain higher residual CF-titres against the group-specific *Bedsonia* antigen. It is well established that individual differences do exist in respect of the capacity to produce and maintain levels of antibody-titres.

The hypothesis offered assumes that the same 'reactive' individuals, if infected with trachoma, manifest also a greater capacity to heal, that is, to develop stage IV trachoma. Consequently, they had to be grouped among the stage IV cases, and this seems to be sufficient to explain the fact that the stage IV cases had a higher mean titre against the group-reactive CF-antigen.

Besides being consistent with all three conclusions (*a*, *b* and *c*) set out above, this hypothesis seems to be supported also by the fact that the existence of individual differences in the capacity to heal spontaneously, that is, to develop stage IV trachoma, has been well established by ophthalmologists (Morax, 1931; Tchirkowsky, 1947; Expert Committee on Trachoma, 1955; Nataf, 1957). The last hypothesis offered calls only for the single assumption that individuals with enhanced capacity to maintain higher residual CF-titres against the group-specific *Bedsonia* antigen, if infected with trachoma virus, manifest also a greater capacity to heal, that is, to develop stage IV trachoma. Whether the association of these two capacities is a cause and effect relation, has no relevance here.

As stated previously, no hypothesis is offered to 'explain' the peculiar observations that in trachoma neither CF, nor CF-inhibiting antibodies do develop against the group-specific *Bedsonia* antigen, which antigen was shown to be produced also by the virus of trachoma.

SUMMARY

Sera of 132 selected patients belonging to stages II, III or IV of clinical trachoma have been tested by CF-test against the ether-soluble, group-specific Bedsonia antigen, as well as by CF-inhibition test against an indicator system of the same group-reactive specificity.

On the basis of the serological results obtained, the following conclusions have been advanced:

Trachoma patients do not develop group-specific CF-inhibiting antibodies in their sera.

The group-reactive (anti-Bedsonia) CF-titres found in sera of trachoma patients represent residual titres developed in response to past infections with agents other than trachoma. There is no satisfactory explanation for the fact that in trachoma patients neither CF, nor CF-inhibiting antibodies develop against the group-reactive Bedsonia antigen, which is known to be produced also by the virus of trachoma.

Statistical analyses revealed that, in the group of stage IV trachomas, the mean values of the anti-Bedsonia CF-titres were significantly higher than those found in the groups of stage II or III, as well as that the factors responsible for that difference must be other than the duration of the trachoma.

A hypothesis was advanced to explain the appearance of the highest group specific mean-titre revealed by the group of stage IV cases.

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