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Antibody Type Specificity to Trachoma in Eye Secretions of Saudi Arab Children

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The presence of trachoma antibody in eye secretions of Saudi Arab children appears to be a direct response to the infecting organism. In 35 of 36 cases, the trachoma antibody type found in eye secretions was identical to the strain type isolated concurrently. Strain types were identified by use of donkey hyperimmune sera prepared against types 1, 1b, and 2. The specificity of the sera was enhanced by absorption with trachoma antigens of heterologous type. Similarly, antibody types were determined by absorbing samples of eye secretions with types 1, 1b, and 2. Complete absorption of trachoma antibody activity was achieved only by the antigen type homologous to the patient's own strain. In one instance, antibody could not be typed. No change in antibody or strain type was seen in four children examined twice during a 3- to 6-month period. Typing of trachoma antibody from eye secretions has potential as an immunological and epidemiological tool.

Antibodies to trachoma were first found in the eye secretions of patients with trachoma by Bernkopf, Orfila, and Maythar (6). In our study of Saudi Arab children (11), a close correlation was found between these antibodies in eye secretions and the clinical and microbiological diagnoses of active trachoma. It was apparent that the presence of antibodies to trachoma in eye secretions in this population was a sensitive and specific test for active trachoma, with potential usefulness as an epidemiological tool.

We have examined 32 Saudi Arab children, from each of whom trachoma strains were isolated and typed. Antibodies to trachoma in eye secretions, obtained at the time of collection of conjunctival scrapings for isolation, were examined for type specificity. The results indicate that eye secretion antibodies have type specificity which correlates well with the antigenic type of trachoma found in the eye.

MATERIALS AND METHODS

Collection of eye secretions and isolation of trachoma agent. The methods used have been described in detail, together with experiments to determine the accuracy of volume estimation of eye secretions and the detection of antibody to two prototype strains of trachoma (11, 12). Fluid, possibly cells, and detritus from the conjunctival sac are herein referred to as eye secretion.

Preparation of typing antisera. Antisera were prepared against three strains of trachoma (12): TRIC/1/ET/HAR-13/OT (formerly Egypt-2), a type 1 prototype strain; TRIC/1b/SAU/HAR-8/OT (formerly SA-8), a type 1b prototype strain; and TRIC/2/SAU/HAR-2/OT (formerly SA-2), a type 2 prototype strain. Adult donkeys were inoculated with a total of 100 to 200 ml of 10% infected crude yolk sac. The inoculum was divided equally, with one half given intravenously and the other given subcutaneously in multiple sites. A second subcutaneous or intravenous dose of similar amount was given with the same material from 41 to 71 days later. The donkeys were bled 2 weeks after the booster, and the sera were preserved with 0.01% thimerosal and stored at −20 C.

Trachoma strains used as absorbing and slide antigens. The prototype strains of 1, 1b, and 2 were used as follows: TRIC/1/ET/HAR-13/OT (12), TRIC/1b/SAU/HAR-32/OT (10), and TRIC/2/SAU/HAR-36/OT (2). These strains were used because they had been cloned by serial limiting dilutions in eggs (S. D. Bell et al., Amer. J. Trop. Med. Hyg., *in press*). Infected crude yolk sacs were diluted in phosphate-buffered saline (*p*H 7.2) to 10 or 50% for use as absorbing antigens and to 1 to 5% before acetone fixation for use as slide antigens.

Typing of trachoma isolates. Type specificity of an antiserum was enhanced by absorption with a cloned trachoma antigen of heterologous type (14, 15). For example, antiserum prepared against HAR-13 (type 1) was diluted 1:8 and absorbed with 3 parts (v/v) of HAR-32 (type 1b) in a 50% yolk sac crude suspension. After 1 hr at 37 C and overnight at 4 C, the absorbed serum reacted only with type 1 antigens, not with types 1b or 2. It was unnecessary to remove the products of absorption prior to testing in the immunofluorescence test. Absorbing ratios of serum to antigen

TABLE 1. *Typing of trachoma strains by use of absorbed hyperimmune donkey sera*[a]

Trachoma strain type[b]	Type 1 antiserum (absorbed with type 1b antigen)[b]	Type 1b antiserum (absorbed with type 1 antigen)[b]	Type 2 antiserum (absorbed with type 1 antigen)
1	+[c]	−	−
1b	−	+	−
2	−	−	+

[a] Type 1 antiserum prepared versus HAR-13; type 1b, versus HAR-8; type 2, versus HAR-2.

[b] Cloned strains: type 1 = HAR-13; type 1b = HAR-32; type 2 = HAR-36.

[c] Positive (+) or negative (−) by immunofluorescence.

varied from 1:1 to 1:3; final dilutions of sera ranged from 1:20 to 1:100. The reaction of these absorbed sera with prototype trachoma antigen is shown in Table 1. The method of typing by indirect immunofluorescence has been described (15).

Typing of antibody in eye secretions. Portions of 0.05 ml of eluted eye secretion were absorbed with 0.01 to 0.05 ml of 10 or 50% infected crude yolk sac suspensions of one of the prototype cloned trachoma strains (HAR-13, HAR-32, and HAR-36) and with uninfected yolk sac.

All measurements were made with 0.05-ml pipettes. Absorptions were carried out in 400-μliter plastic nonwettable tubes at 37 C for 1 hr and overnight at 4 C. Absorbed samples were tested for residual antibody activity to each of the above antigens. The indirect immunofluorescence test was used throughout (11, 15). The conjugate was a commercial anti-human serum globulin prepared in a rabbit and labeled with fluorescein isothiocyanate.

All typing tests of strains or antibodies were coded to prevent bias.

RESULTS

As part of a larger study published earlier (11), 32 Saudi Arab children living in a single village in the Eastern Province were examined in the winter of 1968–1969. Four of the children were seen 3 to 6 months later, for a total of 36 examinations. Trachoma isolation attempts were successful in all 36 examinations, and it was on this basis that these children were selected from the larger study.

Antibodies to prototypes of types 1 and 2 were present in every eye secretion. Sufficient material was present in 27 of 36 specimens to permit titration to an end point; antibody titers ranged from 1:20 to 1:320, with a geometric mean of 1:78.

Trachoma antibody reactivity in an eye secretion was completely absorbed only by antigen of a type homologous with the patient's own strain. Reaction patterns formed by these absorptions are shown in Table 2.

The 36 trachoma strains were typed by use of

TABLE 2. *Reaction patterns used to establish the type specificity of antibodies to trachoma in eye secretions*

Slide antigen[a]	Eye secretions absorbed with				Pattern for antibodies of type
	Trachoma antigens[a]			Uninfected yolk sac	
	Type 1	Type 1b	Type 2		
Type 1	−[b]	+	+	+	
Type 1b	−	−	−	+	
Type 2	−	−	−	+	1
Uninfected yolk sac	−	−	−	−	
Type 1	−	−	−	+	
Type 1b	+	−	+	+	
Type 2	−	−	−	+	1b
Uninfected yolk sac	−	−	−	−	
Type 1	−	−	−	+	
Type 1b	−	−	−	+	
Type 2	+	+	−	+	2
Uninfected yolk sac	−	−	−	−	

[a] Cloned strains: type 1 = HAR-13; type 1b = HAR-32; type 2 = HAR-36.

[b] Positive (+) or negative (−) by immunofluorescence.

hyperimmune trachoma antisera prepared in donkeys and rendered specific by absorption with trachoma antigens of heterologous type. In 35 of the 36 specimens, the type specificity of antibody in eye secretions correlated remarkably well with the type of trachoma strain isolated from the eye of the same patient (Table 3). In one instance, patient 36, absorption was not complete with any antigen.

This was largely a family study. To depict interesting epidemiological aspects, the data are given in detail in Table 3. Of the 36 patients, 22 were siblings in 10 separate families It is noteworthy that in 8 of these 10 families the same type of trachoma agent was found in the eyes of siblings; intrafamily typic variations occurred in only two families. In patients 16 and 17, types 1 and 2 were found in simultaneous examinations of siblings living in the same household; a similar situation was found in patients 21 and 22.

In four children seen twice over an interval of 3 to 6 months, no typic variation in trachoma strain or antibody was found (patients 5 and 6, 7 and 8, 9 and 10, 14 and 15).

The ages of the 32 children ranged from 2 months to 9 years (mean, 3.8 years). Of the 36 patients examined, 30 were diagnosed as active clinical trachoma, 2 were diagnosed as mucopuru-

TABLE 3. *Summary of data from 36 trachoma patients from whom eye secretions for antibody typing were obtained coincident with trachoma isolations*

Patient no.	Clinical diagnosis[a]	Age (years)	No. of inclusions[b]	Trachoma strain designation	Trachoma strain type	Eye secretion antibody		Data from
						Type	Titer[c]	
1	Tr. II	4.7	10	HAR-313	1b	1b	80	Siblings
2	MPC	1.5	71	HAR-359	1b	1b	>5	
3	Tr. III	4.5	0	HAR-361	1	1	>5	Siblings
4	Tr. II	0.7	49	HAR-372	1	1	>5	
5	Hyperemia	2.5	427	HAR-320	2	2	320	Same child
6	Tr. II	3	8	HAR-367	2	2	>5	
7	Tr. II	4	357	HAR-329	2	2	40	Same child[d]
8	Tr. II	4.5	105	HAR-365	2	2	>5	
9	Tr. II	0.2	72	HAR-321	2	2	40	Same child[d]
10	Tr. II	0.7	473	HAR-326	2	2	>10	
11	Tr. II	6	20	HAR-328	1	1	320	Siblings
12	Tr. II	4.5	6	HAR-364	1	1	>10	
13	Hyperemia	1.5	84	HAR-322	1	1	80	
14	Tr. II	5	21	HAR-274	1	1	160	Same child
15	Tr. II	5.5	1	HAR-357	1	1	48	
16	Tr. II	6.5	4	HAR-374	1	1	80	Siblings
17	MPC	1	357	HAR-318	2	2	40	
18	Tr. II	4.5	286	HAR-375	1	1	320	Siblings
19	Tr. II	2.5	16	HAR-284	1	1	40	
20	Hyperemia	0.4	39	HAR-319	1	1	160	
21	Tr. II	9	7	HAR-265	2	2	20	Siblings
22	Tr. II	4	0	HAR-369	1	1	>10	
23	Tr. II	1.5	53	HAR-285	2	2	40	Siblings
24	Tr. II	4.5	377	HAR-272	2	2	80	
25	Tr. II	9	49	HAR-327	2	2	20	Siblings
26	No diagnosis	3.5	134	HAR-371	2	2	80	
27	Tr. II	2	174	HAR-370	2	2	160	
28	Tr. II	4.5	84	HAR-281	1b	1b	20	Siblings
29	Tr. II	2	168	HAR-362	1b	1b	>5	
30	Tr. III	5	1	HAR-360	1b	1b	192	
31	Tr. II	3.5	52	HAR-279	2	2	80	
32	Tr. II	4	28	HAR-277	2	2	80	
33	Tr. II	3	340	HAR-324	1	1	80	
34	Tr. II	5	12	HAR-276	1	1	80	
35	Tr. II	6	92	HAR-269	2	2	80	
36	Tr. III	7	18	HAR-338	2	Untyped	100	

[a] Tr. II or III = trachoma stage II or III, MacCallan classification; MPC = mucopurulent conjunctivitis.

[b] Total number of inclusions in left and right conjunctival scrapings stained by direct immunofluorescence.

[c] Reciprocal of titer by indirect immunofluorescence with the use of homologous antigen.

[d] Siblings.

lent conjunctivitis, and 3 as hyperemia; no diagnosis was made in one patient (Table 3).

Inclusions stained with fluorescent antibody (13) were present in conjunctival scrapings in 34 of 36 examinations; the geometric mean number of inclusions was 37 (Table 3).

DISCUSSION

The presence of antibody type specificity to trachoma in eye secretions has not been described before. Such specificity potentially has considerable immunological and epidemiological significance. The trachoma types described thus far, whether by in vivo (1, 4, 5, 7, 17) or in vitro (3, 10, 15) methods, have been important in cross-protection tests involving toxicity or infection. Homologous challenge of vaccinated mice has shown type-specific vaccine protection against toxic death (1, 4, 5, 7, 17), as well as against pulmonic infection (9). In primates, a relationship between antigenic types and protection from eye infection has been suggested by Wang, Grayston, and Alexander (18). The finding of these workers that challenge with organisms of a heterologous type led occasionally to more severe disease may be even more important, pointing up the possi-

bility that trachoma is, at least in part, an immunopathological disease.

A prior study (11) indicated clearly that antibodies in eye secretions correlated well with clinical and microbiological findings of active trachoma. The present study documents that these antibodies faithfully reflect the type of trachoma organism resident in the eye. The class of antibody produced in the eye in response to the stimulus of the trachoma agent in natural infections in humans (16), in induced eye infections of trachoma in monkeys (16) or guinea pig inclusion conjunctivitis agent in guinea pigs (A. Hathaway and J. H. Peters, Fed. Proc. **28**:766, 1969; F. T. Radcliffe and E. S. Murray, Fed. Proc. **28**:569, 1969) or subsequent to stimulation with killed trachoma antigens given subcutaneously in primates (16) has been described previously. From these studies, it was apparent that both IgG and IgA, the latter presumably secretory in nature, were usually present as antibodies to trachoma in human eye secretions of patients with active clinical trachoma. If the type specificity depicted here resulted only from IgG, specificity in the eye would be due to the previously described specificity of circulating antibody (3, 10, 14, 15) present in the conjunctival sac because of transudation or exudation of serum. If the specificity were found to reside in the secretory IgA molecule as well, then type-specific stimulation and response at the local level may be operative. In either case, the fact that antibodies to trachoma in the eye are type-specific must be taken into account in future laboratory or field studies.

Trachoma has often been described as a family disease, although this concept has been challenged by Woolridge and colleagues (19) and by Detels, Alexander, and Dhir (8). In the village from which the data presented herein were drawn, the three antigenic types of trachoma were found in roughly equal proportions (*unpublished data*). Thus, a random distribution of types of trachoma strains from siblings would have been expected; the finding of similarity in antigenic types in siblings supports the concept of trachoma as a family disease.

Apart from the foregoing, the typing of antibodies in the eye may be a useful epidemiological tool. Microbiological and immunological investigations have a major role in field research in Saudi Arabia. To evaluate trachoma vaccine efficacy during field trials, it has been necessary heretofore to isolate the trachoma organism in eggs to type it, a laborious and time-consuming procedure. If the present study is confirmed, it may be possible in future trials to determine the type of trachoma strain from the antibody in eye secretions, a much simpler task.

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