Immunofluorescence in Chronic TRIC Infections of American Indians and Tunisians: Influence of Trauma on Results of Tests[1] (35333)

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Trachoma is an eye disease which affects about 10% of the world's population. The disease is particularly severe, and often blinding, in countries which are hot and dry. Trauma to the conjunctiva due to dust, hot wind, sand, and other environmental irritants has long been considered to contribute importantly to the prevalence and the severity of trachoma in the hot, arid parts of the Mediterranean basin, Asia, and the southwestern United States.

The clinical disease trachoma results from a composite of influences, including the primary causative agent, *Chlamydia trachomatis* (TRIC agent), repeated superimposed bacterial infections, and a variety of host responses. Direct trauma to ocular structures may influence all of these. In earlier studies we were unable to show that mechanical trauma increased the susceptibility of the conjuctiva to experimental infection with TRIC agents (1). This communication describes the influence of mechanical trauma on preexisting chronic trachoma, as judged by the prevalence of specific immunofluorescence-stained inclusions in conjuctival cells.

Materials and Methods. Individuals. Conjunctival scrapings were collected from groups of school children at 3 boarding schools in the United States and at 1 day school in Tunisia:

1. At Sherman Institute, Riverside, California, there are about 600 students, aged 12–21, from several Indian tribes including Navajo, Apache, Pima, Papago, and others. In March, 1968, as a part of an evaluation of trisulfapyrimidines in the treatment of chronic trachoma (2) scrapings were collected routinely from each of the 31 students in the study. Seven days later a second series of scrapings was collected from the same group of students. One ophthalmologist made all the clinical examinations and collected all the specimens.

2. At Intermountain School, Brigham City, Utah, there are approximately 2000 students, predominantly Navajo, also ranging in age from 12–21. In March, 1969, and at intervals throughout the school year 1969-70, in conjunction with a treatment trial, conjunctival scrapings were collected from randomly selected students with or without active trachoma. On each occasion repeat specimens were collected 1–4 days later. One of 2 ophthalmologists made all the clinical examinations and collected all the specimens.

3. At Stewart School, near Carson City, Nevada, the students also are 12–21 years of age and represent several Indian tribes. Treatment trials have been carried out there during the past several years (2). Paired scrapings were collected from 15 students, representing a range of trachomatous disease during this period.

4. Douz is a small village at an oasis in the southern part of Tunisia, with a high incidence of trachoma. Here children 6–8 years of age, in their first year of elementary school, were seen as a part of a drug evaluation study. Samples were collected in January and in June, 1970 from children who had active disease in October, 1969 and who were in the placebo portion of the study. Conjunctival scrapings were collected by 1 ophthalmologist at an interval of 2 days.

Fluorescence microscopy. All conjunctival scrapings were collected from the upper fornix, using a sterile platinum spatula, several minutes after instillation of an ophthalmic

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surface anesthetic (proparacaine hydrochloride). The removed epithelium was immediately spread on a microscope slide, within an etched circle approximately 1 cm in diameter. Slides were allowed to air-dry, fixed in cold (4°) acetone for at least 15 min, rinsed in phosphate buffered saline, pH 7.1–7.2; again air-dried; and stored at —20° until examined.

The technique for staining and examining the scrapings by immunofluorescence has been described (3, 4). In brief, rabbits without TRIC antibodies were immunized by repeated injection of infected yolk sac material and then bled. Sera selected for high antibody titer by indirect immunofluorescence were precipitated with ammonium sulfate and the globulin fraction was conjugated with fluorescein isothiocyanate. Following chromatography on Sephadex G 50 to remove free fluorescein, the labeled sera were Seitz-filtered, and small aliquots were stored at —20° until used. Evidence for the specificity of these sera for TRIC agents has been presented (3, 5).

All examinations were made by direct immunofluorescence. Scrapings from Sherman Institute and from Stewart School were stained with one single labeled antiserum used in a dilution of 1:20 (4 units); scrapings from Intermountain School and from Douz were stained with another single labeled serum used in a dilution of 1:30 (4 units). All slides were read by the same 2 observers.

Results. Conjunctival trauma markedly influenced both the prevalence of immunofluorescent inclusions and the total number of such inclusions per specimen. Among 31 students at Sherman Institute the increase in numbers of individuals yielding FA+ specimens 7 days after initial trauma was striking ($p<0.001$) as shown in Table I.

At Intermountain School the overall experience was similar: The second of 2 specimens was much more often FA+ than the first. The high prevalence of FA+ inclusions in the first series of specimens collected at

TABLE I. Influence of Trauma on the Prevalence of Immunofluorescent Inclusions in Conjunctival Scrapings.

Geographical location	Examination	Days between scrapings	No. of FA+ individuals/ total no. sampled	% FA+ individuals	
Sherman Institute, California	Mar. '68		11/31	35	$p < 0.001$
	Apr. '68	7	25/31	81	
Intermountain School, Utah	Apr. '69		51/65	78	$p = 0.01$
	Apr. '69	2–4	62/65	95	
	Sept. '69		7/12	58	$p > 0.05$[a]
	Oct. '69	2	9/12	75	
	Jan. '70		2/10	20	$p = 0.05$[a]
	Jan. '70	1	7/10	70	
	Apr. '70		9/28	32	$p > 0.05$[a]
	Apr. '70	1–2	16/28	57	
Stewart School, Nevada	1967–1970		4/15	27	$p < 0.01$
		1–2	13/15	87	
Douz School, Tunisia	Jan. '70		4/24	17	$p < 0.001$
	Jan. '70	2	20/24	83	
	June '70		1/24	4	$p = 0.05$
	June '70	2	7/24	26	
Total	1st Exam		89/209	42	$p < 0.001$
	2nd Exam		159/209	76	

[a] If the results from specimens Sept. '69 to Apr. '70 are combined, 18/50 are FA+ in the first examination and 32/50 are FA+ in the second examination, yielding $p = 0.01$.

TABLE II. Influence of Trauma on the Number of Immunofluorescent Inclusions in a Given Individual.

Geographic location	Examination	Days between scrapings	Prevalence of FA+ inclusions/examination				Percentage of persons with increased FA+ inclusions in 2nd exam
			1st < 2nd	1st = 2nd	1st > 2nd	Total	
Sherman Institute, California	Mar.–Apr. '68	7	19	12	0	31	61
Intermountain School, Utah	Apr. '69	2–4	47	11	7	65	72
	Sept. '69	2	8	3	1	12	67
	Jan. '70	1	5	5	0	10	50
	Apr. '70	1–2	13	13	2	28	47
Stewart School, Nevada	1967–1970	1–2	12	3	0	15	100
Douz, Tunisia	Jan. '70	2	19	5	0	24	79
	June '70	2	6	17	1	24	25
Total	1967–1970	1–7	129	69	11	209	62

Intermountain School in April, 1969 may indicate a spread of infection, perhaps also reflected by a flurry of active cases in the school during that particular spring. In the 3 later examinations at this school, numbers were smaller, and the interval between the first and second examinations shorter. Nevertheless, there was a marked increase of FA+ inclusions in the second specimens. At Stewart School on 15 occasions repeat specimens were collected at 1–2 day intervals, again with a significant increase in FA+ prevalence.

In Tunisia, in January, 1970, where the second scrapings were collected 48 hr after the first, the results are comparable to those from students at Sherman Institute ($p < 0.001$). In the same population in June, 1970 the total number of inclusions demonstrated was too small to permit a significant difference.

The average scraping examined in our laboratory contains 5000–10,000 epithelial cells. We have felt that true quantitation is impossible, because of variations in the numbers of cells per specimen and in the occurrence of clumps of cells and mucus in the specimens which make meaningful counts difficult. However, the numbers of *typical intracytoplasmic inclusions* in each scraping has been recorded, and the number in the first specimen compared with the number in the second, as shown in Table II. Among 209 individuals included in this study, 129 (62%) showed an increased number in the second examination, while in only 11 (5.3%) was there a decrease. The pattern of increased numbers in the second specimen remained constant for each of the subsamples described.

There were only a few typical immunufluorescent inclusions in any 1 initial scraping. For example, of the individuals at Sherman Institute, 11/31 yielded FA+ scrapings initially, and in those a total of 14 FA+ inclusions were seen (1–3/positive scraping; calculated mean = 1.3). After trauma, 25 of the 31 yielded FA+ scrapings, and a total of 107 FA+ inclusions were seen (1–13/positive scraping, calculated mean = 3.1). Of 24 individuals at Douz, in January 1970, 4 yielded FA+ scrapings initially with a total of 5 FA+ inclusions (1–2/positive scraping, calcu-

TABLE III. Influence of the Time Interval Between Scrapings on the Increased Prevalence of Immunofluorescent Inclusions.

	Days elapsed between first and second scrapings									
	1		2		3		4		7	
Geographic location	No.	%	No.	%	No.	%	No.	%	No.	%
Sherman Institute, California									19/31	61
Intermountain School, Utah	8/21[a]	38[b]	18/29	62	11/13	85	27/41	66		
Stewart School, Nevada	7/9	79	5/6	84						
Douz School, Tunisia			26/48	54						

[a] Number of individuals showing increased prevalence of immunofluorescent inclusions in the second scraping/total number of individuals examined.

[b] Percentage of individuals showing increased prevalence of immunofluorescent inclusions in the second scraping.

lated mean = 1.3). After trauma 20 were FA+, with a total of 171 FA+ inclusions (1–23/positive scraping, calculated mean = 6.0).

The time elapsed between the first and the second scraping may have some influence upon the results of the second examination. All repeat specimens at Sherman Institute were collected after an interval of 7 days, and all at Douz after an interval of 2 days. Only at Intermountain School was a direct comparison of time intervals possible. It appears that scrapings collected 2 to 4 days after the initial trauma are most likely to show increased prevalence of FA+ inclusions (Table III). On 1 occasion 2 scrapings were collected at intervals of 5 min from each of 20 individuals. No increase in prevalence of FA+ scrapings or in the number of FA+ inclusions in the second scraping was noted.

Discussion. In our hands, immunofluorescence has provided specific laboratory evidence of TRIC agent infection when other procedures (Giemsa stains of conjunctival scrapings, isolation of agent in yolk sac or cell culture, complement fixation tests) have failed (3–5). Even with this technique, we have demonstrated FA+ inclusions in only 45–65% of individuals with clinically active disease, and the technique has remained relatively insensitive. In addition, adhering to the criteria of this laboratory, and in the populations we have studied, only small numbers of typical FA+ inclusions are to be found in positive smears. Any modification of the technique increasing the sensitivity of the test without decreasing its specificity would be of great value. Traumatizing the conjunctiva by a preliminary scraping appears to have this effect. The striking increase in the prevalence of FA+ specimens, together with the consistent increase in numbers of inclusions per scraping suggests a specific relationship between trauma and prevalence of immunofluorescent inclusions.

All examinations described here were part of clinical surveys of larger groups, and the second specimens were usually collected on the last day of the examination period. For this reason we have no definite information regarding possible changes in disease picture which might result from the trauma.

While our data do not permit adequate evaluations of the relative effectiveness of the various time intervals between collection of specimens, it appears that 2–4 days are optimal for the effect of trauma, and up to 7 days acceptable. Obviously, as a part of field studies, the shorter interval is more practical.

In view of the data presented here, it is interesting to reconsider the "provocative tests" with cortisone administration in trachoma. Ormsby *et al.* (6) referred to "repeated" scrapings over a period of 2 weeks, but did not specify exact time or relationship to cortisone treatment. Freyche *et al.* (7) examined clinically and microbiologically, every 48 hr, their series of 50 "healed" trachoma patients, and reported activation of disease with reappearance of Giemsa-

staining inclusions in 13/50, or 26%. Mohsenine and Darougar (8) selected 25 individuals who were considered "cured." At the time steroid therapy was begun, all were scraped and were Giemsa-negative. Inclusions reappeared in 8/25 patients on day 3 of therapy, in 14/25 on day 6, and in 16/25 on day 9. It is tempting to suggest that the daily conjunctival scraping contributed to the reactivation.

At the present time we have no explanation for the mechanism of the increased prevalence of immunofluorescent inclusions following trauma. It might be a local, mechanical effect in bringing infected cells to the surface, chlamydial replication might be enhanced, or some systemic effect might be at work.

Summary. The conjunctivas of 209 individuals from endemic trachoma areas in the southwestern United States and in Tunisia were scraped. One to 7 days later they were scraped again. Both series of conjunctival scrapings were examined by fluorescent microscopy. Both the prevalence of FA+ specimens in the populations studied, and the number of FA+ inclusions in inclusion-positive scrapings increased markedly in the second specimen, following trauma. We conclude that trauma in some manner enhances the presence of immunofluorescent inclusions in chronic TRIC infections.

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