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Immunotypes of *Chlamydia trachomatis* Isolates in Seattle, Washington

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The immunotype distribution of 493 *Chlamydia trachomatis* isolates from 467 adults and 26 infants in the Seattle area from 1965 to 1982 is presented. All except one of the isolates from adults were from the genitourinary tract, rectum, or bubo. The proportions of each immunotype were ED, 46.5%; GF, 24.6%; H, I, J/CJ, and K, 5 to 7% each; B, 3.5%; and L_2, 1%.

A new classification separates *Chlamydia trachomatis* species into three biovars: trachoma, lymphogranuloma venereum (LGV), and mouse (N. R. Krieg, ed., Bergey's Manual of Systematic Bacteriology, in press). Trachoma and LGV biovars are natural pathogens for humans. The main disease syndromes caused by these pathogens are conjunctival, respiratory, and genitourinary infections. The trachoma biovar is further classified into 12 (A through K plus Ba) serovars (immunotypes) and the LGV biovar into three (L_1, L_2, and L_3) serovars by the microimmunofluorescence test (1, 9, 14–16). Over the past 18 years, we immunotyped more than 1,000 *C. trachomatis* isolates from all over the world. In 1977 we reported the immunotyping of 560 isolates, including 100 from Seattle (17). Since then, we immunotyped 400 additional Seattle isolates. Seattle isolates are referred to as UW (University of Washington) strains in our publications. They constitute the largest collection of typed strains from a single geographical area.

This study spanned the period from June 1965 to September 1982 at the affiliated hospitals of the University of Washington in Seattle, the University Hospital, Harborview Medical Center, and Seattle Public Health Hospital (formerly the U.S. Public Health Hospital). The great majority of the adult patients came from the sexually transmitted disease clinic at Harborview Medical Center. The adult females also included prenatal care patients. The disease syndromes associated with *C. trachomatis* infection are nongonococcal urethritis, Reiter's syndrome, proctitis, and buboes in adult males, symptomatic (mucopurulent cervicitis) and asymptomatic cervical infection, urethral syndrome, endometritis, salpingitis, Fitz-Hugh and Curtis syndrome (peritonitis and perihepatitis), and proctitis in adult females, and conjunctivitis, rhinitis, otitis media, and pneumonitis in infants.

Several culture systems were used for isolation of the strains. Inoculation was done in chicken embryo yolk sac cultures (13) until January 1969 and in DEAE-dextran-pretreated HeLa 229 cell cultures (10) after June 1971. Some isolations were done in X-ray-irradiated (4), 5-iodo-2-deoxyuridine-treated (19), or cycloheximide-treated (11) McCoy cell cultures. In the beginning, the isolates were immunotyped by the mouse toxicity prevention test (1), which was changed to the standard two-way microimmunofluorescence test (14) in 1971, and finally to the simplified one-way micro-immunofluorescence test (18) after 1973. The Ba antigen is not incorporated in the one-way test because it gives a broad serological cross-reaction with other B-complex antigens (B, Ba, D, E, L_1, and L_2). Therefore, including it in the one-way test tends to obliterate the separation of B-complex immunotypes.

A total of 532 isolates from 490 patients were immunotyped. Table 1 shows the immunotype distribution of 493 isolates by the anatomical sites of the samples obtained from adults and infants. When multiple isolates were obtained from different sites for the same patient, the first isolate or the one with a higher titer was usually selected for immunotyping. When more than one isolate was immunotyped for females, the cervical isolate was used for tabulation. When series of isolates from the same patient were immunotyped, the strains were entered only once if the immunotype remained the same. The immunotypes of the second isolates were different from those of the first for only three patients.

The trachoma biovar was isolated from the cervix, urethra, endometrium, salpinx, rectum, and throat in females; the urethra and rectum in

TABLE 1. Immunotype distribution of 493 *C. trachomatis* isolates from 490 patients in Seattle

Immunotype[a]	No. of isolates from culture site[b]:													Total no. isolated (%)
	Female adults						Male adults			Infants				
	Cx	Ur	Em[c]	Sx	Rt	Th	Ur	Rt	Bu	Ey	NP	Rt	Vg	
B	9	2					4	1		1				17 (3.5)
D	45	3	1			1[d]	18	1			1		1	70 (14.2)
E	34	4					7	1		3	1	1	1	52 (10.6)
ED	59	12	1		4		14	1		5	2		1	98 (19.9)
BED	7									1	1			9 (1.8)
F	54	6	1		2		14	3		1	1	1		82 (16.6)
G	9	1					8	1						19 (3.9)
GF	11	1					8							20 (4.1)
H	22	2		1	1		4			1				31 (6.3)
I	25	4					5			1				35 (7.1)
J	11	2	1				3							16 (3.3)
CJ	10													10 (2.0)
K	24						3			2				29 (5.9)
L_2								3	2					5 (1.0)

[a] Immunotypes A, Ba, C, L_1, and L_3 were not isolated.
[b] Cx, Cervix; Ur, urethra; Em, endometrium; Sx, salpinx; Rt, rectum; Th, throat; Bu, buboes; Ey, conjunctiva; NP, nasopharynx; Vg, vagina.
[c] Endometrial isolates were not included in the tabulation; the companion cervical isolates were used instead.
[d] Isolated from a 29-year-old female with upper respiratory infection who had had a spontaneous abortion 3 weeks before.

males; and the conjunctiva, nasopharynx, rectum, and vagina in infants. The LGV biovar was isolated from the rectum and buboes. All the immunotypes of the trachoma biovar except A, C, and Ba have been found in Seattle. Whether types C and Ba are present in Seattle is unknown, because Ba antigen was excluded from the test and the serologically close relationship of C and J makes it difficult to differentiate them in the one-way test. The only LGV immunotype found was L_2. It is not always possible to differentiate between the closely related immunotypes D and E, B, E, and D, G and F, or C and J by the one-way test. In these cases, the immunotypes were designated ED, BED, GF, and CJ.

Of the trachoma biovar isolates, 46.5% were type ED; 24.6% were GF; 5 to 7% each were H, I, J/CJ, or K; and 3.5% were B (Table 1). The immunotypes of isolates from sexual partners were usually the same, as were those of infants and their mothers. Of 14 pairs of sexual partners, 12 pairs (89%) had the same immunotypes when the partners were tested within 1 month of each other, as did the five infant-mother pairs. Except for LGV immunotypes, no preferential association of immunotype and disease syndrome was seen. Of the 14 Reiter's syndrome isolates, 6 were ED, 6 were GF, 1 was B, and 1 was J. Of 12 rectal isolates from proctitis patients (10 males and 2 females), 9 were the trachoma biovar, with 4 type ED, 4 type GF, and 1 type B, and 3 were the LGV biovar, all type L_2. Of four cervical isolates from patients with Fitz-Hugh and Curtis syndrome, three were type ED and one was type F.

Isolates from all except one of the adults were from the genital tract. All isolates from infants, regardless of the anatomical site of isolation, could be regarded as being of genital origin because the infant was presumably infected in the genital tract of the mother. The immunotype distribution of these genital isolates was similar to that of those from other geographical areas (17) and to that of the small number of isolates typed by another group of investigators (3) (Table 2).

Among the 28 LGV strains (8 from San Francisco, 6 from Washington, D.C., 5 from Seattle, 5 from Ethiopia, 3 from Australia, and 1 from London) that we typed, the distribution was 3 (11%) type L_1, 21 (75%) type L_2, and 4 (14%) type L_3.

So far, we have not identified type A, Ba, or C trachoma biovar strains in Seattle. Type Ba has been associated with classical trachoma in North American Indians (5, 17). However, we found four genital-type Ba isolates in Denmark. Types A, B, Ba, and C are generally associated with endemic trachoma in the Far East, Middle East, and North Africa (5, 17), although type D was isolated from trachoma patients in both Africa and Taiwan.

This observation caused the misleading classification of the trachoma biovar into ocular or trachoma (A, B, Ba, and C) and genital (D through K) immunotypes (3, 12). Although the presence of types A and C of genital origin has

TABLE 2. Immunotype distribution of genital *C. trachomatis* isolates in other geographical areas

Geographical area (strain code)	Investigator (reference)	No. of isolates	No. (%) of isolates that were immunotype:		
			ED	GF	Other[a]
Seattle (UW)	This study	493	229 (46.5)	121 (24.6)	143 (29.0)
Denmark (DK)[b]	C. H. Mordhorst	47[c]	28 (59.6)	10 (21.3)	9 (19.2)
Finland (FI)[b]	P. Saikku	51	29 (56.9)	7 (13.7)	15 (29.4)
Europe	Dwyer et al. (3)	23	13 (56.5)	9 (39.1)	1 (4.4)

[a] Other immunotypes were: B, H, I, CJ, K, and L_2 (Seattle); Ba, H, J, and K (Denmark); B, H, I, J, and K (Finland); and L_3 (Europe).

[b] These isolates were typed by us.

[c] Included ocular isolates of apparent genitoocular transmission.

not been reported in the United States, type B strains constituted 3.5% of all of our genital isolates.

Definable microbiological and virulence markers for differentiating ocular and genital strains were not found. Our pathogenesis studies with monkey eye inoculation showed that all trachoma serovars cause follicular conjunctivitis in monkeys similar to human eye infection, although genital isolates tended to cause more severe symptoms and a longer course of eye infection in monkeys than did ocular isolates from areas of endemic trachoma (2, 9, 15, 16). Two genital isolates from Seattle (serovars D and G) and two ocular trachoma isolates (serovars B and C) from Taiwan showed similar pathogenesis in our mouse lung model (7). We also compared the growth of trachoma biovar strains in HeLa cell and chicken embryo yolk sac cultures (8). We found that the HeLa cell culture was equal to or greater than the yolk sac culture in sensitivity. Furthermore, ocular and genital isolates differed in their ability to propagate in egg and cell cultures: genital strains grew much better in the cell culture, but ocular strains grew only slightly better in the cell culture. The geographical distribution of immunotypes seen today could have been determined by thousands of years of selective pressure by socioeconomic and environmental factors rather than by differences in the pathogenic characteristics of the immunotypes.

Because the treatment of *C. trachomatis* infection is not affected by the immunotype of the infecting organisms except for the LGV biovar, and because immunotyping is expensive and time-consuming, we would not recommend it as a routine procedure. However, immunotyping is useful for epidemiological studies and for tracing an infectious source. Whenever possible, an isolate from a newly discovered disease syndrome or from a case in which the etiology is in question should be immunotyped.

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