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Genetic Variability among *Chlamydia trachomatis* Reference and Clinical Strains Analyzed by Pulsed-Field Gel Electrophoresis

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Pulsed-field gel electrophoresis (PFGE) was applied to *Chlamydia trachomatis* reference strains representing each of the 18 serovars and to 29 clinical isolates from genital specimens collected in Bordeaux, France, or Malmö, Sweden. Comparison of the fingerprint patterns of the reference strains revealed a high level of polymorphism of the total DNA when *Sma*I was used (14 profiles), whereas the other enzymes, *Sse*8387I and *Apa*I, showed fewer differences. Some serovars, considered to be closely related on the basis of their antigenic determinants located on the major outer membrane protein (MOMP), such as D and Da or I and Ia, were shown to be different after PFGE of their genomic DNAs. However, serovars B and Ba and serovars L2 and L2a had identical patterns after analysis with the three endonucleases. When applied to clinical isolates, which were typed by restriction fragment length polymorphism analysis of the MOMP gene, PFGE allowed the detection of intragenotype polymorphisms and showed the identity of two strains successively isolated from the same patient. This technique seems to be an efficient tool for epidemiological studies when used in addition to serotyping or genotyping by restriction fragment length polymorphism analysis of the MOMP gene.

The genus *Chlamydia* contains four species, *Chlamydia trachomatis*, *Chlamydia psittaci* (19), *Chlamydia pneumoniae* (14), and the newly described *Chlamydia pecorum* (11). The *C. trachomatis* species is divided into three biovars; one biovar is pathogenic for mice, and two biovars, lymphogranuloma venereum (LGV) and trachoma biovars, are pathogenic for humans (19). Human isolates are classified into 18 serovars on the basis of serological variations of the major outer membrane protein (19, 31). Serovars L1, L2, L2a, and L3 (LGV biovar) are the agents of lymphogranuloma venereum, a sexually transmitted disease that is rare in developed countries. The trachoma biovar is composed of serovars A, B, Ba, and C, which are responsible for trachoma, which is endemic in developing countries, and serovars D through K, which are worldwide causes of oculogenital and neonatal infections.

Typing of *C. trachomatis* is commonly performed by serotyping (32). Recently, molecular methods based on the nucleotide variations of *ompl*, the gene encoding the major outer membrane protein (9, 12, 23, 25), have been used. Although D, I, and L2 genovariants and serovariants have been described (8, 18, 22, 26), genovariants of serovars E (26), F, and G, the more commonly encountered serovars, have rarely or never been detected.

Restriction fragment length polymorphism (RFLP) analysis of genomic DNA detected by pulsed-field gel electrophoresis (PFGE) is an efficient tool for the genomic characterization of bacteria or viruses and for epidemiological studies. PFGE has recently been applied to *C. trachomatis* for the construction of a physical map of the L2 serovar strain and the evaluation of its genome size (3). In the investigation described here, we applied PFGE to the 18 *C. trachomatis* reference strains in an attempt to compare the LGV and trachoma biovars as well as strains belonging to each serovar. In addition, the PFGE technique was extended to clinical isolates with the *ompl* genotypes, which are more frequently encountered in genital infections (22), to determine genomic variations, which should allow for more discriminant epidemiological studies and strain identification, especially with strains isolated from the same patient.

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MATERIALS AND METHODS

***C. trachomatis* strains.** The *C. trachomatis* strains analyzed in the study were reference strains representing each of the 18 serovars and clinical isolates. Reference Da (TW-448), Ia (UW-202), and L2a (UW-396) strains were obtained from the Washington Research Foundation (Seattle, Wash.), and the 15 other strains, which have been described previously (23), were obtained from the American Type Culture Collection. The 29 clinical strains, belonging to the D, Dv, E, F, and G genotypes, were isolated from genital specimens; 6 strains were kindly given by K. Persson (Malmö General Hospital, Malmö, Sweden), and 23 strains were isolated in Bordeaux, France. *C. trachomatis* reference and clinical strains were genotyped by *ompl* RFLP analysis after amplification by PCR by following a previously described method routinely used in our laboratory (22, 23) that allows the determination of the Dv strains as *ompl* genovariants of the D strains.

EB purification. Each chlamydial strain was grown on McCoy cell monolayers in six flasks (75 cm^2) at 37°C for 48 to 72 h with 5% CO_2. Elementary bodies (EBs) were purified by a previously described protocol (30). Briefly, cell debris was eliminated by centrifugation at 2,000 × *g* for 15 min. EBs were recovered by centrifugation for 40 min at 20,000 × *g*. The pellet was suspended in 1 ml of 50 mM Tris-HCl (pH 7.5)–10 mM $MgCl_2$. A DNase I (Sigma Chemical Co.) treatment (50

μg/ml) was performed for 2.5 h at 37°C to digest the remaining eukaryotic DNA. Bacteria were collected by centrifugation at 20,000 × *g* for 40 min, and the pellet was then suspended in phosphate-buffered saline (PBS; pH 7.2). Quantifications were performed by diluting the bacterial suspensions in PBS and counting the organisms after staining with a direct fluorescent monoclonal anti-*C. trachomatis* antibody (MicroTrak; Syva, San Jose, Calif.). Pellets of purified EBs from each strain were then stored at −20°C until they were used to prepare agarose blocks. Uninfected McCoy cell monolayers were treated by the same protocol described above to verify the efficiency of the EB purifications. This preparation was used as a control of McCoy DNA contamination of the EB preparation.

DNA preparation. DNA preparation was performed by the method described by Schwartz and Cantor (28). EBs were suspended in 10 mM Tris-HCl (pH 8.0)–50 mM EDTA (pH 8.0) to about 2×10^{10} per ml, mixed with an equal volume of 1% low-melting-point agarose (SeaPlaque; FMC Corp), and dispensed in a slot former of 100 μl. The agarose blocks were incubated for 1 h at 37°C in 0.5 M EDTA (pH 8.0) and then for 24 h at 56°C after the addition of 1% (wt/vol) of *N*-lauroyl sarcosine (Sigma) and 1 mg of proteinase K (Boehringer, Mannheim, Germany) per ml. Proteinase K was inactivated by washing the blocks two times in 20 ml of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0]) containing 3.5 mg of phenylmethylsulfonyl fluoride (Boehringer) for 1 h at 37°C. The buffer was changed, and the blocks were washed for 1 h at room temperature and then three times in 20 ml of TE buffer. The McCoy cell DNA preparation (control of EB purification) was also embedded in agarose blocks and was treated similarly. In some experiments McCoy cell DNA, obtained after sodium dodecyl sulfate (SDS)-proteinase K treatment of the cells and embedded in agarose blocks, was used as a control.

Restriction endonuclease analysis of DNA. Agarose blocks containing about 1 μg of chlamydial DNA were subjected to restriction endonuclease digestions. Before DNA hydrolysis, inserts were equilibrated in 600 μl of the respective enzyme buffers for 1 h at 4°C. Restriction enzymes were added after elimination of about 500 μl of 1× buffer. For complete digests of DNAs, 10 U of *Asc*I, 8 U of *Not*I (Biolabs Biochemical Inc.), 40 U of *Apa*I, 30 U of *Sfi*I, 20 U of *Sgr*AI, 25 U of *Sma*I (Boehringer), 20 U of *Sse*8387I (Amersham, Takara Biochemical Inc.), or 20 U of *Eco*RI (Bethesda Research Laboratories) was used. After an overnight incubation at 4°C, digestions were conducted for 6 h at 50°C for *Sfi*I and 37°C for the other enzymes. Bovine serum albumin (0.01%; wt/vol) was added to the *Not*I buffer, and the digestion was performed for 18 h at 37°C. Blocks of McCoy cell DNA were also digested in parallel.

Gel electrophoresis. The DNA restriction fragments were separated either by conventional 0.8% agarose gel electrophoresis (24), after *Eco*RI digestion, or with a contour-clamped homogeneous field electrophoresis system (CHEF-DRII or CHEF-DRIII; Bio-Rad Laboratories) after digestion with the other enzymes. PFGE was performed in 0.5× TBE buffer (44.5 mM Tris-HCl, 44.5 mM boric acid, 1 mM EDTA [pH 8.0]) at 8°C. One percent agarose gels (Appligene) were subjected to electrophoresis for 18 h at 190 V, with pulse times ranging from 10 to 30 s. A bacteriophage lambda concatemer (Bio-Rad) was used as a molecular size marker. The gels were stained in the running buffer containing 0.5 μg of ethidium bromide per ml, rinsed, and photographed under UV light.

Southern transfer. The agarose gels were soaked in 0.25 M HCl for 20 min, and the DNA was denatured in 0.4 M NaOH and transferred overnight onto GeneScreen filters (DuPont de Nemours) with 0.4 M NaOH (24). The filters were washed in 2× SSC (1× SSC is 150 mM NaCl plus 75 mM sodium citrate [pH 7.0]). DNA was cross-linked to the filters by exposure to UV light (312 nm, 2 min).

DNA probe hybridizations. Two different DNA probes were used: purified McCoy cell DNA and *omp1*-amplified fragments. Prehybridizations were performed at 65°C for 2 h, by following the manufacturer's instructions for GeneScreen filters, in 10% (wt/vol) dextran sulfate–1% (wt/vol) SDS–1 M NaCl–500 μg of sonicated and denatured herring sperm DNA per ml. To control the efficiency of the EB purification step, purified McCoy cell DNA, obtained after SDS-proteinase K treatment of the cells and phenol-chloroform extraction (24), was used as the probe after labeling by nick translation with [α-^{32}P]dCTP (ICN Biomedicals, Orsay, France) on the *Eco*RI restriction fragments of the 18 *C. trachomatis* reference strains. *omp1*-amplified fragments from strains from serovars D, E, F, and L2, obtained by PCR with the CT1 and CT5 primers (23), were mixed and purified on cDNA spun columns following the manufacturer's instructions (Pharmacia). The CT1-CT5-amplified fragments (0.1 μg) were labeled with [α-^{32}P]dCTP (ICN) by random priming (24) and were used as probes on the *Sma*I restriction DNA fragments of the 18 *C. trachomatis* reference strains. Hybridizations were carried out overnight at 65°C. The filters were washed twice in 2× SSC containing 0.1% SDS at room temperature for 5 min and were washed twice in 0.1× SSC containing 0.1% SDS for 15 min at 65°C. The filters were exposed to X-Omat AR films (Eastman Kodak Co.) for 24 h.

RESULTS

Digestion by a high-frequency-cleavage restriction endonuclease. Analysis of *Eco*RI digestions of the DNAs from the 18 reference strains on conventional agarose gels showed indistinguishable restriction patterns (data not shown), with a large number of fragments of less than 20 kbp. Hybridization of these fragments with the total McCoy cell DNA showed a low level of eukaryotic DNA contamination in the chlamydial EB preparations.

Choice of suitable restriction endonucleases. Because the G+C content of *C. trachomatis* is considered to be 42 to 45% (7, 17), seven restriction endonucleases recognizing eight- or six-bp sequences or G+C rich sites (1) were tested for their suitability for use in PFGE by using a single *C. trachomatis* strain (data not shown). The experimentally suitable endonucleases generating between 15 and 25 fragments were *Sse*8387I (CCTGCAGG), as shown in a previous study (3), *Apa*I (GGGCCC), and *Sma*I (CCCGGG). The restriction profiles of *Asc*I (GGCGCGCC), *Not*I (GCGGCCGC), *Sfi*I (GGCCN$_5$GGCC), and *Sgr*AI (C(A/G)CCGG(T/C)G) digestions clearly showed only a few fragments and were more appropriate for use in genome mapping or size evaluation studies of *C. trachomatis*.

Application to the 18 *C. trachomatis* reference strains. DNAs from purified EBs of the 18 reference strains were digested with three endonucleases, *Sma*I, *Apa*I, and *Sse*8387I, and were separated by PFGE. The results of these three digestions are summarized in Table 1.

The restriction patterns obtained with *Sma*I are presented in Fig. 1A. The *Sma*I digestion revealed a large polymorphism in the 18 *C. trachomatis* strains, generating 14 different profiles numbered M1 to M14 (Table 1). With the exception of strains A and C (M1 profile) and B and Ba (M2 profile), all of the trachoma biovar strains could be differentiated by their *Sma*I profiles. All of the trachoma biovar strains presented a specific

TABLE 1. RFLP profiles of the 18 reference strains on total DNA by PFGE

Serovar (reference strain no.)	*ompl* genotype	Biovar[a]	Profile no.			
			*Sma*I	*Apa*I	*Sse*8387I	*ompl* probe hybridization
A (Har-13)	A	TR	M1	A1	S1	I
B (Har-36)	B/Ba	TR	M2	A1	S2	I
Ba (AP-2)	B/Ba	TR	M2	A1	S2	I
C (TW-3)	C	TR	M1	A1	S1	I
D (UW-3)	D	TR	M3	A2	S3	II
Da (TW-448)	Da	TR	M4	A3	S4	III
E (Bour)	E	TR	M5	A3	S4	IV
F (IC-Cal-3)	F	TR	M6	A3	S4	IV
G (UW-57)	G	TR	M7	A2	S5	I
H (UW-43)	H	TR	M8	A2	S6	I
I (UW-12)	I	TR	M9	A2	S7	II
Ia (UW-202)	Ia	TR	M10	A2	S8	I
J (UW-36)	J	TR	M11	A2	S9	I
K (UW-31)	K	TR	M12	A2	S10	II
L1 (440)	L1	LGV	M13	A4	S11	II
L2 (434)	L2	LGV	M14	A5	S11	IV
L2a (UW-396)	L2a	LGV	M14	A5	S11	IV
L3 (404)	L3	LGV	M13	A4	S11	II

[a] Abbreviations: TR, trachoma; LGV, lymphogranuloma venereum.

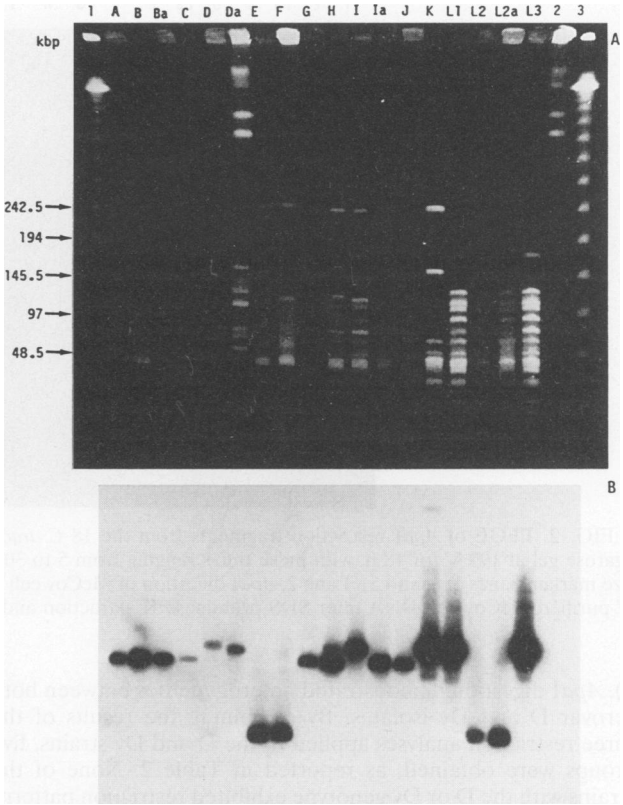

FIG. 1. PFGE of *Sma*I restriction fragments from the 18 *C. trachomatis* reference strains (A). The digestion products were separated on a 1% agarose gel at 190 V for 18 h, with pulse times ranging from 5 to 30 s in 0.5× TBE. Bacteriophage lambda concatemers were used as molecular size marker (lanes 1 and 3). Lane 2, McCoy cell DNA prepared following the EB purification protocol. (B) Fingerprint patterns obtained after Southern blot hybridization of the α-^{32}P-labeled *ompl* probe to the *Sma*I fragments.

240-kbp fragment (Fig. 1A, serovars A to K) that was never found in the LGV biovar strains, which allowed for the differentiation of the two biovars. Closely related patterns were obtained for the H and I serovars, whereas the Ia serovariant strain seemed to have fewer *Sma*I sites, which explained the lower number of fragments. Resemblances in the restriction profiles were also observed for serovars E, G, and K, but these strains could always be differentiated. Among the LGV biovar strains, both L1 and L3 and both L2 and L2a had identical restriction patterns (M13 and M14, respectively) and could not be differentiated from each other. The M13 and M14 profiles differed only by an additional 150-kbp fragment. The McCoy cell DNA gave four fragments after *Sma*I digestion. These fragments were larger than 400 kbp and so could not interfere with the chlamydia-specific restriction profiles, especially in the Ba, Da, and F preparations that exhibited contamination by McCoy cell DNA (Fig. 1A).

*Apa*I digestion generated five different patterns (Fig. 2), which were composed of fragments smaller than 140 kbp. The LGV biovar strains (L1 to L3) again differed from the trachoma biovar strains, giving two specific profiles (A4 and A5). Serovar A, B, Ba, and C strains gave a specific A1 profile with a 110-kbp fragment, while serovar D, G, H, I, Ia, J, and K strains had a fragment of approximately 120 kbp (A2 profile). *Apa*I digestion of Da, E, and F strains generated a completely different pattern (A3 profile). The McCoy cell DNA control gave very large fragments. McCoy cell DNA contamination did not interfere with the interpretation of the chlamydia-specific *Apa*I patterns.

The *Sse*8387I restriction profiles of the 18 serovars are shown in Fig. 3 and are reported in Table 1. The McCoy cell DNA control presented several restriction fragments after *Sse*8387I digestion, especially two fragments of about 160 and 70 kbp that could interfere with the interpretation of the chlamydia-specific restriction profiles. Thus, differentiation of the restriction profiles from the chlamydial reference strains was done without taking into account fragments of 160 and 70 kbp. The LGV strains differed from the trachoma biovar strains by exhibiting an S11 profile (Fig. 3). The A and C serovar strains had an identical pattern with a 200-kbp fragment (S1 profile). The S2 profile, specific to serovar B and Ba strains, showed two bands of about 190 and 210 kbp, while the other fragments were smaller than 100 kbp (Fig. 3). The 10 other trachoma strains exhibited eight different profiles (S3 to S10), with a common S4 pattern for the Da, E, and F serovars (Fig. 3).

Combining the results of the three digestions did not increase the number of different profiles detected after *Sma*I treatment among the 18 reference strains of *C. trachomatis*.

Application to clinical strains. Genotyping of the 29 clinical isolates selected for the study by PCR and RFLP gave the following results: 13 E strains (6 from Malmö, Sweden, and 7 from Bordeaux, France), 4 F strains, 3 G strains, 5 D strains, and 4 strains genotyped as Dv (22). The DNAs of the 29 strains were digested with *Sma*I, and 25 of these 29 strains were studied by *Apa*I and *Sse*8387I digestions. The *Sma*I restriction profiles of most of these strains are shown in Fig. 4. The total results of the three digestions are reported in Table 2.

*Sma*I analysis of the total DNA gave a distribution of D and Dv strains identical to that obtained after *ompl* genotyping (22, 23). The five D and the four Dv clinical strains showed M3 and M6 profiles, respectively. *Sse*8387I digestion showed a homogeneity among the D isolates, whereas two groups were identified among the Dv-genotyped strains (Fig. 5 and Table

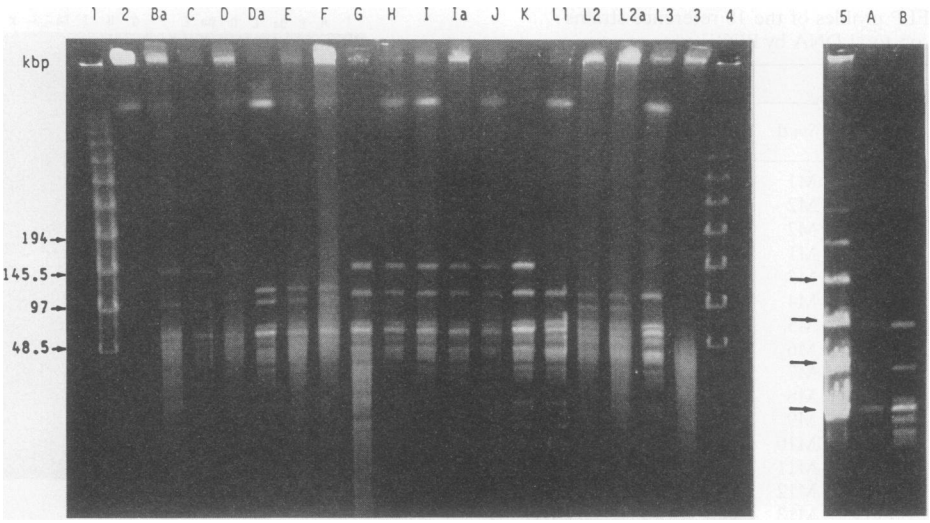

FIG. 2. PFGE of *Apa*I restriction fragments from the 18 *C. trachomatis* reference strains. The digestion products were separated on a 1% agarose gel at 190 V for 18 h, with pulse times ranging from 5 to 30 s in 0.5× TBE. Bacteriophage lambda concatemers were used as molecular size marker (lanes 1, 4, and 5). Lane 2, *Apa*I digestion of McCoy cell DNA prepared following the EB purification protocol; lane 3, *Apa*I digestion of purified McCoy cell DNA after SDS-proteinase K extraction and ethanol purification.

2). *Apa*I digestion demonstrated heterogeneities between both serovar D and Dv isolates. By combining the results of the three restriction analyses applied to the D and Dv strains, five groups were obtained, as reported in Table 2. None of the strains with the D or Dv genotype exhibited restriction patterns completely identical to those of the serovar D or Da reference strains, M3/A2/S3 and M4/A3/S4, respectively. Among the four Dv strains analyzed, two of them (183/Bx and 309/Bx) were isolated from the same patient within a 15-month period. The total RFLP analysis (Fig. 5) by PFGE identified the two isolates as identical strains.

An intragenotype *Sma*I polymorphism was observed for the E-genotyped strains (Fig. 4, lanes 15 to 23), distributing these strains into two groups. The first group was characterized by an M5 profile (Fig. 3, lane 17) for two strains (identical to that of the serovar E reference strain), and the 11 other strains showed an M6 profile (identical to that of the serovar F reference strain). Furthermore, the same heterogeneity was

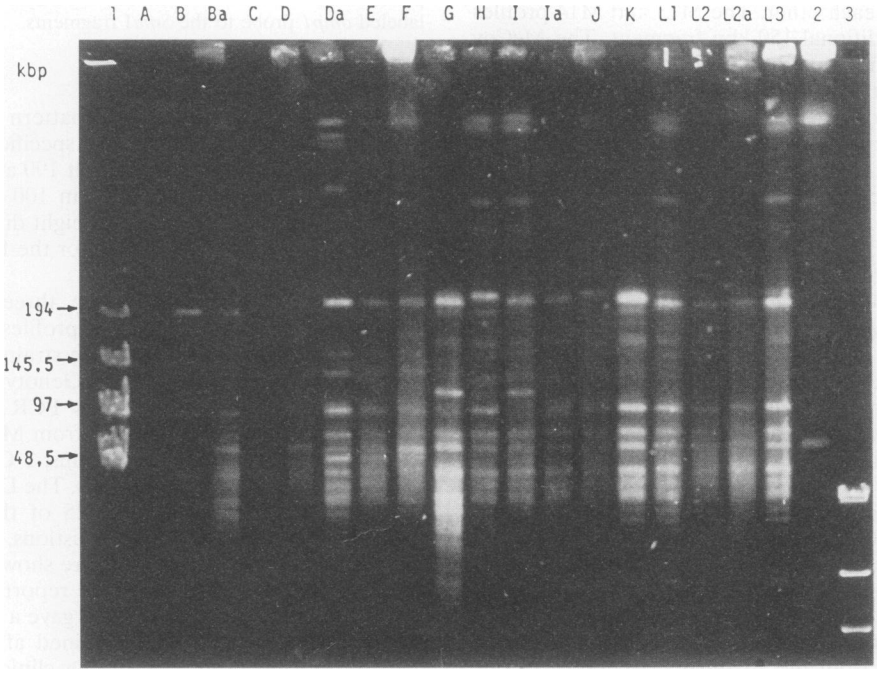

FIG. 3. PFGE of *Sse*8387I restriction fragments from the 18 *C. trachomatis* reference strains. The digestion products were separated on a 1% agarose gel at 190 V for 18 h, with pulse times ranging from 5 to 30 s in 0.5× TBE. Bacteriophage lambda concatemers (lanes 1 and 2) and lambda DNA cleaved by *Eco*RI (lane 3) were used as molecular size markers.

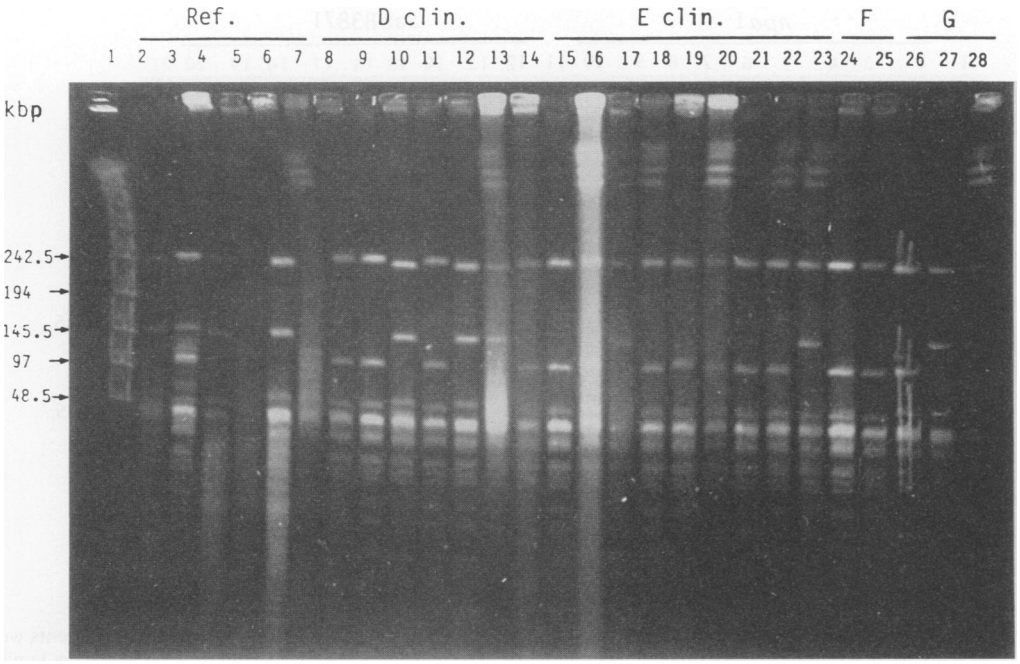

FIG. 4. PFGE of *Sma*I restriction fragments from 6 reference strains and 21 clinical isolates. The restriction fragments were separated on a 1% agarose gel at 190 V for 18 h, with pulse times ranging from 5 to 30 s in 0.5× TBE. Bacteriophage lambda concatemers were used as molecular size markers (lane 1). Lanes 2 to 7, reference strains of serovars D (lane 2), Da (lane 3), E (lane 4), F (lane 5), G (lane 6), and L2 (lane 7); lanes 8 to 28, clinical isolates of the D serovar (lanes 8 to 14), the E serovar isolated in Bordeaux (lanes 15 to 18) or Malmö (lanes 19 to 23), the F serovar (lanes 24 and 25), and the G serovar (lanes 26 to 28).

observed among E strains isolated in Bordeaux or Malmö (Table 2). The *Apa*I and *Sse*8387I profiles of all of the E isolates were identical to those of the serovar E reference strain.

Among the four F clinical strains, an intragenotype polymorphism was observed for one strain (234/Bx) after *Apa*I and *Sse*8387I digestions (Table 2). The three other strains showed an M6/A3/S4 pattern identical to that of the serovar F reference strain.

An intragenotype polymorphism was detected for two of the three G clinical strains after *Sma*I and *Sse*8387I digestions (Table 2). None of the three strains presented a complete restriction profile identical to that of the serovar G reference strain (M7/A2/S5).

By combining the results of the three digestions, new profiles different from those of the reference strains were obtained among the D, E, F, and G clinical strains.

Localization of *ompl* on DNA from reference strains. The CT1-CT5-amplified fragment of *ompl* was used as a probe on Southern blots of the *Sma*I digests from all 18 reference strains. Figure 1B presents the hybridization patterns found with the reference strains. Because only one band was detected after hybridization, the gene was found to be present in all of the strains, probably in a single copy. The sizes of the hybridized fragments were different among the strains, ranging from about 20 to 45 kbp. Four main hybridization groups (groups I to IV) were produced as shown in Table 1. The 18 strains were distributed among the four groups without any differentiation of LGV and trachoma biovar strains.

DISCUSSION

The polymorphism of the total DNA of *C. trachomatis* strains has been studied by a number of techniques, including

TABLE 2. RFLP profiles of 29 clinical isolates on total DNA by PFGE

Strain no.	*ompl* genotype	Profile no.		
		*Sma*I	*Apa*I	*Sse*8387I
143/Bx[a]	D	M3	A1	S10
197/Bx	D	M3	A2	S10
188/Bx	D	M3	A2	S10
235/Bx	D	M3	A2	S10
242/Bx	D	M3	A2	S10
183/Bx	Dv	M6	A3	S1
186/Bx	Dv	M6	A6	S11
202/Bx	Dv	M6	A3	S11
309/Bx	Dv	M6	A3	S1
17030/Mal[b]	E	M6	ND[c]	ND
17165/Mal	E	M6	ND	ND
17549/Mal	E	M6	ND	ND
17623/Mal	E	M6	A3	S4
17778/Mal	E	M6	A3	S4
17854/Mal	E	M5	A3	S4
151/Bx	E	M6	A3	S4
192/Bx	E	M6	A3	S4
199/Bx	E	M6	A3	S4
209/Bx	E	M6	A3	S4
212/Bx	E	M5	ND	ND
214/Bx	E	M6	A3	S4
237/Bx	E	M6	A3	S4
127/Bx	F	M6	A3	S4
184/Bx	F	M6	A3	S4
234/Bx	F	M6	A2	S5
241/Bx	F	M6	A3	S4
185/Bx	G	M10	A2	S5
190/Bx	G	M7	A2	S1
210/Bx	G	M7	A2	S1

[a] Bx, designated Bordeaux as the origin of the strain.
[b] Mal, designated Malmö as the origin of the strain.
[c] ND, not done.

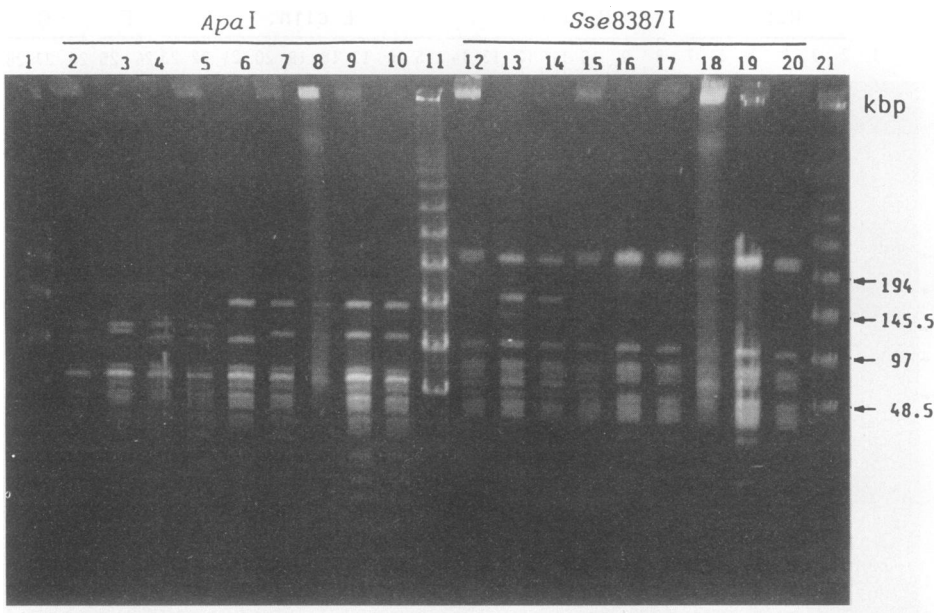

FIG. 5. PFGE of *Apa*I and *Sse*8387I restriction fragments of the nine D or Dv clinical isolates. The restriction fragments were separated on a 1% agarose gel at 190 V for 18 h, with pulse times ranging from 5 to 30 s in 0.5× TBE. Lanes 1, 11, and 21, bacteriophage lambda concatemers; lanes 2 and 12, strain 183/Bx; lanes 3 and 13, strain 186/Bx; lanes 4 and 14, strain 202/Bx; lanes 5 and 15, strain 309/Bx; lanes 6 and 16, strain 143/Bx; lanes 7 and 17, strain 235/Bx; lanes 8 and 18, strain 242/Bx; lanes 9 and 19, strain 188/Bx; lanes 10 and 20, strain 197/Bx.

restriction enzyme analysis (20), ribotyping, hybridization of randomly cloned DNA probes (30), and random amplification of polymorphic DNA (RAPD) (29). All of those studies demonstrated intraserovar heterogeneities with total DNA among clinical isolates from some serovars, but never among serovar F strains. DNA fingerprinting analyzed by PFGE, another technique for investigating total DNA, has been used for the physical study of the *C. trachomatis* serovar L2 strain genome (3, 10) and has been widely used for epidemiological studies on several bacteria (1, 2, 4–6, 13, 21, 27), but to our knowledge it has never been used on *C. trachomatis* clinical isolates.

The analysis of the 18 *C. trachomatis* reference strains by PFGE showed heterogeneity between strains of the trachoma and LGV biovars and between most of the different serovars, despite DNA homologies of nearly 100% (7, 19) observed after DNA-DNA hybridization. Digestion with three different enzymes clearly separated the LGV biovar strains from those belonging to the trachoma biovar. The two biovars of human strains are differentiated on the basis of their pathogenic and growth characteristics (19). PFGE showed that the strains belonging to the two biovars also differ in their total DNA sequences. Similar results were obtained by Peterson and de la Maza (20), who found that *Eco*RI digestion of total DNA from several *C. trachomatis* strains gave a specific pattern for the LGV strains. Our findings, extended to 18 reference strains, confirm those previous data. In the same way, by using PFGE, the A to C serovar strains could be differentiated from the D to K serovar strains and from the LGV strains when they were studied by *Sma*I and *Sse*8387I digestion. Scieux et al. (29) reported a similar differentiation of the *C. trachomatis* reference strains by RAPD analysis.

Fourteen total restriction patterns were determined with the 18 *C. trachomatis* reference strains with the three enzymes used in the study. The *Sma*I restriction profiles revealed a greater polymorphism than those obtained after *Sse*8387I or *Apa*I digestions. PFGE applied to the *C. trachomatis* reference strains is less discriminant than serotyping or *ompl* genotyping. These results are in agreement with the data presented by Kaltenboeck et al. (16), who reported that the serovars of *C. trachomatis* strains that infect humans have virtually identical genomes but highly divergent *ompl* loci. The 18 reference serovars were determined on the basis of the antigenic diversity of the major outer membrane protein (19, 31) or genomic variations of the *ompl* gene (8, 33). The *ompl* genomic variation also allowed Yuan et al. (33) to subdivide the human reference strains into three groups: the B complex (B, Ba, D, E, L1, L2), the C complex (A, C, H, I, J, K, L3), and the intermediate group (F, G). This distribution was not found after PFGE analysis of the total DNA of the reference strains; for example, closely related *Sma*I profiles were obtained for the E, G, and K serovar strains, whereas each of these strains belongs to a different *ompl* group. On the contrary, the B and L2 serovar strains exhibited total PFGE profiles identical to those of their respective serovariants, Ba and L2a, which differ from each other by only a few nucleotides in their *ompl* sequences (33). The lack of correlation between the B and C complexes, the intermediate group, and PFGE genotypes could be due to differences in the *Sma*I, *Apa*I, or *Sse*8387I restriction sites outside of *ompl*.

Localization of *ompl* among the *Sma*I fragments revealed that the 18 reference strains fell into four main groups. Each strain within each of the four groups exhibited only one hybridization band. This result confirms the absence of an *Sma*I recognition site inside the *ompl* sequence, as shown by the nucleotide sequences reported by Yuan et al. (33). However, the 18 strains were distributed into these four groups without any correlation with the PFGE profiles, the pathogenicities, or any other characteristics of the strains.

Within each of the D, Dv, E, F, and G clinical isolates, it was possible to detect polymorphisms with one or two enzymes. More heterogeneities were detected in the D- and Dv-geno-

typed strains than within strains of the three other serovars. Five groups were determined among nine serovar D and Dv strains, allowing an exact identification of the clinical isolates. Eleven of the 13 E clinical isolates varied from their serovar reference strain in their *Sma*I profiles. The two remaining strains, 17854/Mal and 212/Bx, were isolated in Malmö and Bordeaux, respectively. Because of the random selection of the 29 clinical isolates included in the study, the two *Sma*I patterns of the E strains cannot be a geographical artifact but may represent two groups found in the two countries. Polymorphisms detected on the total DNA from *C. trachomatis* clinical isolates have been described previously among serovar D, E, and G strains (20, 29, 30) but never among serovar F strains. Using PFGE, we detected one polymorphic F strain. The 29 clinical isolates examined in the present study were of the D, Dv, E, F, and G *ompl* genotypes, which were responsible for more than 80% of the *C. trachomatis* infections in a recent study performed in Bordeaux (22). Interestingly, these 29 strains could be separated into four groups by serotyping, into five groups by *ompl* genotyping, and into 11 groups by PFGE. This result shows the more discriminative power of the PFGE technique compared with that of serotyping or *ompl* genotyping for the epidemiological study of *C. trachomatis* infections.

Interestingly, all of the different PFGE profiles determined for the clinical isolates with each of the three endonucleases individually were previously detected in one of the reference strains that sometimes belonged to another serological group. Thus, the PFGE patterns of clinical isolates do not correlate with those of the serovar or *ompl* sequence variants. Similar findings were reported for leptospiral strains, demonstrating divergences between the classifications obtained by the serological technique and PFGE (15).

In the present study, PFGE was shown to be an efficient tool for the identification of the *C. trachomatis* strains, especially when the technique was applied on clinical strains of the same serovar or *ompl* genotype and successively isolated from the same patient. Our data revealed a genomic polymorphism between most of the 18 reference strains and among strains with the same *ompl* genotype, but also the genomic stabilities of clinical strains isolated from different geographical sites. Although the technique is more labor-intensive, it reduces the complexity of the restriction endonuclease analysis fingerprinting patterns, thereby facilitating comparisons between strains. PFGE can be an interesting alternative for the complete identification of *C. trachomatis*, but it cannot replace serotyping or *ompl* genotyping. Furthermore, determination of total genomic variants between clinical isolates of the same serovar or the same *ompl* genotype could provide interesting information about their genetic structures.

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