INFECTION AND IMMUNITY, Aug. 1983, p. 604–608
0019-9567/83/080604-05$02.00/0

Vol. 41, No. 2

Characterization of *Chlamydia* DNA by Restriction Endonuclease Cleavage

ELLENA M. PETERSON* AND LUIS M. DE LA MAZA

Pathology Department, University of California, Irvine, California 92717

Received 17 January 1983/Accepted 11 May 1983

The DNA from six serovars of *Chlamydia trachomatis*, lymphogranuloma venereum (LGV) I, LGV II, LGV III, B, C, and D, and from *Chlamydia psittaci* was extracted, treated with restriction endonuclease enzymes, and run on agarose gels. By using this technique, the DNA of *C. trachomatis* could be clearly differentiated from *C. psittaci* DNA. A comparison of the DNA from the different serovars of *C. trachomatis* revealed similar patterns with and without detectable differences. LGV I, LGV II, LGV III, B, and C revealed no differences when treated with *Bam*HI, *Hae*III, *Xba*I, and *Xho*I. LGV III DNA, when cleaved with *Eco*RI and *Hha*I, had a major band migrating faster than the other two LGV serovars. Serovar D had a different pattern from all other strains tested when cleaved with *Bam*HI, *Eco*RI, *Hha*I, *Hin*cI, and *Xho*I. When treated with *Sac*I and *Hga*I, LGV II displayed a unique band not seen in the other LGV serovars. Differences in strains could be attributed to both chromosomal and plasmid DNA.

In the past 10 years, *Chlamydia trachomatis* has been recognized as a leading sexually transmitted pathogen as well as a causative agent of pneumonia and conjunctivitis in newborns (12). There are 15 serovars of *C. trachomatis*; however, it appears that antigenically there is a great amount of cross-reactivity among all strains of *C. trachomatis* (15). The serovars can be separated into three groups based on their pathogenic potential: lymphogranuloma venereum (LGV) strains LGV I, LGV II, LGV III; trachoma strains, A, B, Ba, and C; and genital strains D through K (13).

Although this organism is an important human pathogen, little is known about its DNA (1). Reports to date have described molecular weight estimations (6, 11, 14), structural characterization (11), DNA hybridization for relatedness (7), and plasmid content of the genus *Chlamydia* (9). With increasing awareness of its pathogenicity, it is important to determine the genetic makeup of this organism. By studying the DNA of *Chlamydia* spp., we might gain insight into the underlying reason for the apparent differences in pathogenicity among strains, an understanding of the possible mechanisms for antimicrobial resistance, and a better epidemiological understanding of the infections caused by Chlamydia spp.

In this study using six serovars of *C. trachomatis*, we employed restriction endonucleases to characterize the DNA from all three pathogenic groups and from *Chlamydia psittaci*.

MATERIALS AND METHODS

Organisms. *C. trachomatis* strains and *C. psittaci* VR-601 were obtained from the American Type Culture Collection, Rockville, Md., and J. Schachter (Hooper Foundation, San Francisco, Calif.). *C. trachomatis* serovars included in this study were LGV I (strain 440), LGV II (strain 434), LGV III (strain 404), B (strain TW-1), C (strain TW-3), and D (strain IC-Cal-8). Organisms received in egg yolk were subsequently passaged in HeLa 229 and McCoy cells. Cells were infected by centrifuging (1,000 × *g* and 30°C) the inoculum onto the host monolayer for 1 h, after which Eagle minimal essential medium with 5% fetal bovine serum, gentamicin (10 μg/ml), and cycloheximide (1 μg/ml) were added (10). Cells were incubated at 37°C for 48 to 72 h.

DNA labeling and isolation. For DNA labeling, 100 μCi of $^{32}P_i$ was added to phosphate-free Eagle minimal essential medium with 5% fetal bovine serum 20 h after the infection of a 150-cm^2 flask of HeLa cells. Forty-eight hours after infection the cells were scraped into 2 ml of 10 mM Tris-hydrochloride (pH 7.5)–50 mM EDTA–150 mM NaCl and disrupted by vortexing with glass beads. When approximately 90% of the cells were broken, the suspension was centrifuged at 500 × *g* for 5 min; the supernatant was collected and centrifuged in an SW41 rotor at 15,000 rpm for 30 min at 4°C. The resulting pellet was suspended in 10 mM Tris-hydrochloride (pH 7.5)–150 mM NaCl and centrifuged as before. The pellet was digested with pronase (500 μg/ml) and sodium dodecyl sulfate (0.5% [wt/vol]) at 37°C overnight, followed by dialysis against 10 mM Tris-hydrochloride (pH 7.5)–150 mM NaCl–15 mM sodium citrate–5 mM EDTA. Cesium chloride was added to a density of 1.7060 g/cm^3, and the lysates were centrifuged at 22°C for 24 h in a VTi-65 vertical rotor

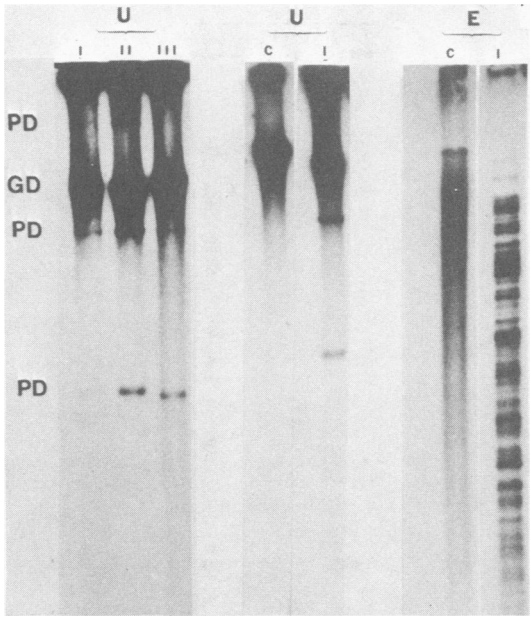

FIG. 1. Agarose gel (1%) containing DNA extracted from uninfected HeLa 229 cells (C) and HeLa 229 cells infected with LGV I (I), LGV II (II), and LGV III (III). Preparations shown are undigested (U) and digested with *Eco*RI (E). Bands in the undigested preparations include genomic DNA (GD) and plasmid DNA (PD).

(Beckman Instruments, Inc.) at 40,000 rpm. Peak fractions which corresponded to a density of 1.7060 to 1.7063 g/cm^3 were pooled and dialyzed. RNase (50 μg/ml) was added and incubated for 1 h (37°C), followed by two extractions with phenol and chloroform-isoamyl alcohol (24:1). The DNA was precipitated and rinsed with cold ethanol.

Plasmid isolation in sucrose gradients. The DNA preparation from the cesium chloride gradient was collected and dialyzed, and the DNA was layered on top of a 5 to 20% (wt/vol) sucrose gradient as previously described (2). The gradients were centrifuged for 3 h at 39,000 rpm at 20°C with an SW41 rotor in a model L5 preparative Beckman ultracentrifuge. The gradients were collected from the bottom of the tube, and the samples were counted with a Beckman LS900 liquid scintillation counter.

Preparation of herpes simplex virus type 1 (HSV-1) DNA. HSV-1 (strain F) was obtained from the American Type Culture Collection and grown in Vero cells, using $^{32}P_i$ for labeling. The DNA was extracted and purified by the method described by Hirt (5). The nomenclature and molecular weight values used were those reported by Hayward et al. (4) and Locker and Frenkel (8).

Restriction enzyme treatment and electrophoresis. Samples were divided, and the optimum buffer and salt concentration for the particular restriction enzyme (New England Biolabs, Beverly, Mass.) were used. Enzymes were added to achieve complete digestion, and reactions were incubated for 3 h at 37°C. In some instances in which DNA digests appeared different, enzyme was added every 8 h up to 24 h to check for complete digestion. Bromophenol blue containing glycerol was added to each reaction, and samples were loaded onto agarose gels. After electrophoresis, gels were dried, exposed, and developed.

RESULTS

Uninfected HeLa cell control DNA is shown in Fig. 1. In the undigested reaction there is a band of high-molecular-weight chromosomal DNA. Undigested *Chlamydia* DNA preparations, obtained from all three LGV types (I, II, and III) grown in HeLa cells, are also shown in Fig. 1. Each LGV strain has a main chromosomal component and three additional well-defined bands which represent plasmid DNA. *C. psittaci* and the other *C. trachomatis* serovars tested, B, C, and D, also contain plasmid DNA. The different plasmid bands probably represent large aggregates and closed and nicked circles.

As expected, upon digestion with *Eco*RI, HeLa cell DNA did not generate a set of well-defined DNA fragments. In contrast, well-defined DNA fragments were generated from LGV I-infected HeLa cells (Fig. 1). McCoy cells were also infected with LGV to rule out the possibility that HeLa DNA or other contaminating DNA

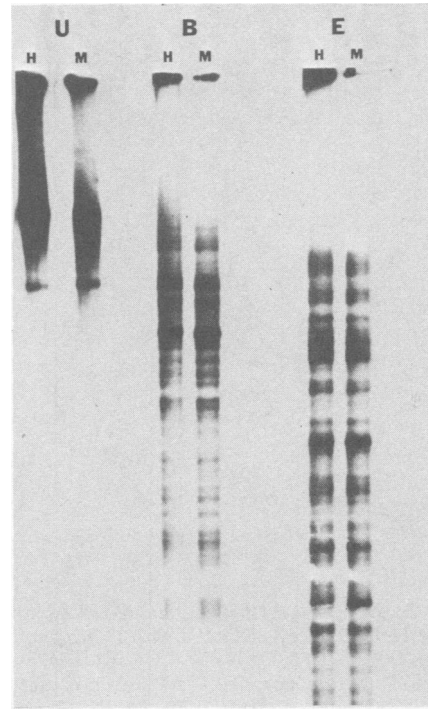

FIG. 2. DNA extracted from LGV II-infected HeLa 229 (H) and McCoy (*M*) cells. DNA shown was undigested (U) and digested with *Bam*HI (B) and *Eco*RI (E) and run on agarose gels (2%).

was isolated from the infected preparations. Undigested and *Eco*RI- or *Bam*HI-digested DNA from LGV II-infected HeLa or McCoy cells generated identical gel patterns, adding further evidence that the digests were *Chlamydia* DNA (Fig. 2).

Upon digestion with all restriction enzymes tested, there were significant differences between the DNA fragments generated from *C. trachomatis* and *C. psittaci* (Fig. 3). On the other hand, when *C. trachomatis* strains were cleaved with restriction enzymes and their fragments compared, small or no differences were found among the digests. When cleaved with *Bam*HI, *Eco*RI, *Hae*III, *Hha*I, *Xba*I, and *Xho*I, serovars LGV I, LGV II, LGV III, B, and C revealed minimal differences. An example of a cleavage reaction with no apparent DNA fragment differences is shown in Fig. 3 with *Hae*III. Similar patterns with a change in migration rate in a band are shown in Fig. 3 and 4 with *Eco*RI and *Hha*I. In these two examples, it is LGV III that has one faster major band.

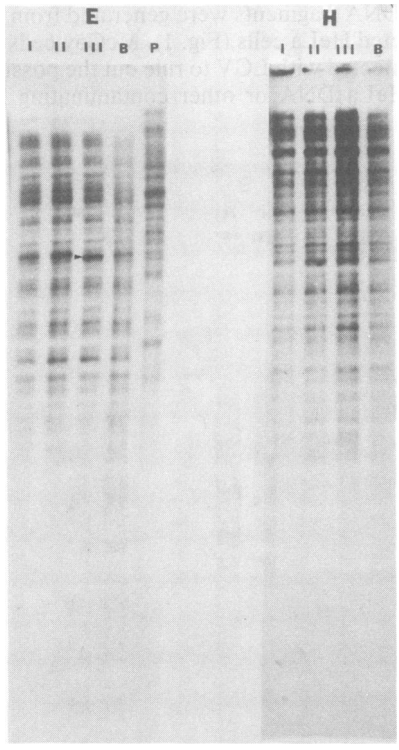

FIG. 3. Agarose gel (1%) containing DNA extracted from HeLa 229 cells infected with *C. trachomatis* serovars LGV I (I), LGV II (II), LGV III (III), and B and with *C. psittaci* (P). The DNA shown is digested with *Eco*RI (E) and *Hae*III (H). The *Eco*RI and *Hae*III digests are from two separate gels run on different days. No distinct differences in the DNA fragment patterns can be seen, with the exception of the migration rate of a major band (arrow) of LGV III.

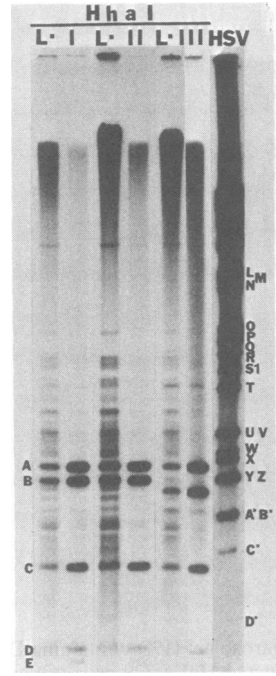

FIG. 4. Cleavage of chromosomal and plasmid DNA from LGV I, LGV II, and LGV III with *Hha*I. HSV-1 (strain F) was cleaved with *Bam*HI as a molecular weight marker. The left lane from each serovar represents the digestion product of the total elementary body DNA, whereas the right lane shows the cleavage of the purified plasmid DNA.

When LGV I and D were run in parallel after digestion with *Bam*HI, *Eco*RI, *Hha*I, *Hin*cI, or *Xho*I, there were detectable differences. Examples are shown in Fig. 5 with *Bam*HI, *Eco*RI, and *Hha*I. Also, when the three LGV serovars were cleaved with *Hga*I and *Sac*I there was a band present in LGV II that was not detected in the other two serovars (Fig. 5).

A plasmid was isolated from the main *Chlamydia* genome by cesium chloride and sucrose gradients and subsequently cleaved with *Bam*HI, *Eco*RI, *Sac*I, and *Hha*I. When HSV-1 digests were used as a molecular weight marker (Fig. 4 and 6), the size of the plasmid was estimated to be 4.5×10^6 daltons. In Fig. 6 it is clear that cleavage with *Bam*HI and *Sac*I generated linear DNA, and a comparison of the three LGV serovars showed similar migration rates. Therefore, the band difference seen with LGV II DNA cleaved with *Sac*I (Fig. 5) cannot be attributed to a plasmid difference but must be a difference in the main chromosome. However, when the plasmid from the LGV serovars was cleaved with *Eco*RI (Fig. 6) and *Hha*I (Fig. 4),

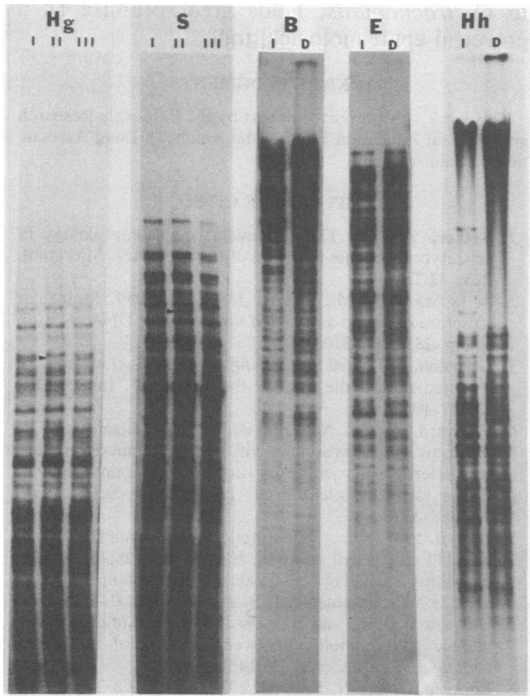

FIG. 5. Agarose gel (1%) displaying the DNA from HeLa 229 cells infected with LGV I (I), LGV II (II), LGV III (III), and D. Differences in the strains (arrows) can be seen with all enzymes, *Sac*I (S), *Hga*I (Hg), *Bam*HI (B), *Eco*RI (E), and *Hha*I (Hh). Gels of the different enzymes were run on different days with several DNA preparations.

the LGV III plasmid had a faster migrating *Eco*RIA (Fig. 6) and *Hha*IB fragment (Fig. 4). These results paralleled those of the total *Chlamydia* DNA cleavage with *Eco*RI and *Hha*I in which one LGV III band differed in migration. Therefore, in these cases it was the cleavage of the plasmid and not the main chromosomal DNA that resulted in a distinctive digestion pattern.

DISCUSSION

To date there have been only a few reports on *Chlamydia* DNA. Tamura (14) reported a molecular weight of 950×10^6 for *C. psittaci* DNA. By analysis of the rate of reassociation of denatured DNA, Kingsbury (6) estimated that the genome of *C. psittaci* contained 8.5×10^5 nucleotide pairs and that of *C. trachomatis* contained 6.0×10^5. Sarov and Becker (11), using a gentle technique for releasing the DNA, determined that the molecular weight of *C. trachomatis* DNA was 660×10^6 based on their analysis by centrifugation in sucrose gradients and electron microscopy. Kingsbury and Weiss (7), using DNA hybridization techniques, found only a 10% degree of relatedness between the two species of *Chlamydia*, but the three strains of *C. trachomatis* they used could not be differentiated.

In this study we attempted to differentiate species and strains of *Chlamydia* by DNA restriction endonuclease patterns. With this treatment there were marked differences in the DNA of *C. psittaci* and *C. trachomatis*. However, the DNA from the serovars of *C. trachomatis* tested, LGV I, II, and III, B, C, and D, were all very closely related but differed slightly, depending on the restriction enzyme used. Differences were found in the main genomic DNA and in the plasmid present in all strains.

The 15 serovars of *C. trachomatis* can be separated into three groups on the basis of their pathogenic spectrum. These groups are the LGV strains, I, II, and III; the trachoma group, A, B, Ba, and C; and the genital strains, D through K (13). By using microimmunofluorescence it appears that certain serovars within a pathogenic

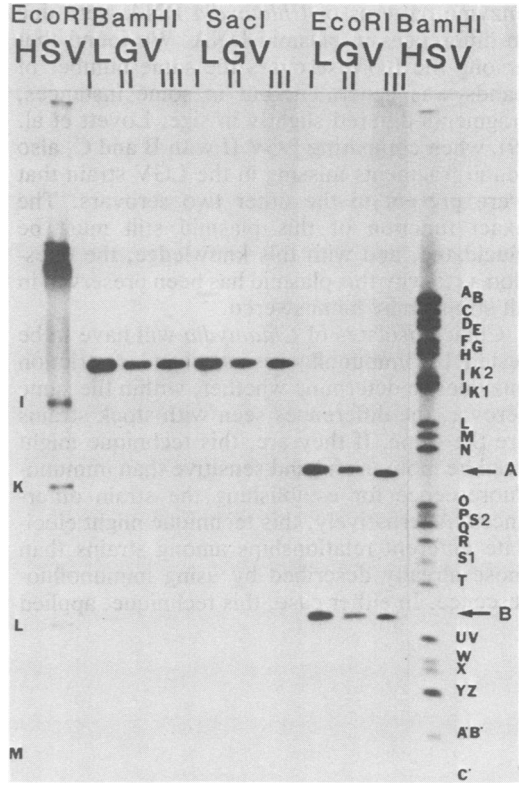

FIG. 6. Cleavage of plasmid DNA from LGV I, LGV II, and LGV III with *Bam*HI, *Sac*I, and *Eco*RI. After digestion of DNA with restriction enzymes, the reactions were electrophoresed in a 1% horizontal agarose gel. HSV-1 (strain F) cleaved with *Eco*RI or *Bam*HI was used as a molecular weight marker. The figure is a composite of two different exposure times of the same gel.

group are more closely related to serovars in other groups rather than to those of similar pathogenic spectrum. Grayston and Wang (3), using immunofluorescence, have described the B complex, which is composed of serovars B, Ba, D, E, LGV I, and LGV II. Not only is there a great amount of cross-reactivity among all members of this complex, but within the complex, E-D and LGV I-LGV II can only be differentiated with a two-way cross test of both antigen and antibody. From the enzymes we used it appeared that LGV I and B were more closely related than LGV I and LGV II; D differed from all other members of the complex tested; and LGV III was more closely related to members of the complex than D. However, more extensive testing of strains by this method must be done before the complex relationship among the serovars can be clarified.

It is interesting to note that both species of *Chlamydia* and all six *C. trachomatis* serovars tested had a plasmid(s) with a similar molecular weight. Some of the differences in the restriction enzyme patterns of *Chlamydia* DNA were due to differences in plasmid DNA. We found that among the LGV serovars the same number of bands was generated, but in some instances, fragments differed slightly in size. Lovett et al. (9), when comparing LGV II with B and C, also found fragments missing in the LGV strain that were present in the other two serovars. The exact function of this plasmid still must be elucidated, and with this knowledge, the question as to why this plasmid has been preserved in all strains may be answered.

Clinical isolates of *Chlamydia* will have to be tested by immunofluorescence and restriction enzymes to determine whether, within the same serovar, the differences seen with stock strains are the same. If they are, this technique might even be more useful and sensitive than immunofluorescence for establishing the strain differences. Alternatively, this technique might elucidate different relationships among strains than those already described by using immunofluorescence. In either case, this technique, applied to *C. trachomatis*, holds great promise as a powerful epidemiological tool.

ACKNOWLEDGMENTS

This work was supported in part by the California Research and Medical Education Fund of the American Lung Association of California.

LITERATURE CITED

1. **Becker, Y.** 1978. The chlamydia: molecular biology of procaryotic obligate parasites of eucaryocytes. Microbiol. Rev. **42**:274–306.
2. **de la Maza, L. M., and B. J. Carter.** 1980. Molecular structure of adeno-associated virus variant DNA. J. Biol. Chem. **255**:3194–3203.
3. **Grayston, J. T., and S. P. Wang.** 1975. New knowledge of chlaymdiae and the disease they cause. J. Infect. Dis. **132**:87–105.
4. **Hayward, G. S., N. Frenkel, and B. Roizman.** 1975. Anatomy of herpes simplex virus DNA: strain differences and heterogeneity in the locations of restriction endonuclease cleavage sites. Proc. Natl. Acad. Sci. U.S.A. **72**:1768–1772.
5. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
6. **Kingsbury, D.** 1969. Estimate of the genome size of various microorganisms. J. Bacteriol. **98**:1400–1401.
7. **Kingsbury, D. T., and E. Weiss.** 1968. Lack of deoxyribonucleic acid homology between species of the genus *Chlamydia*. J. Bacteriol. **96**:1421–1423.
8. **Locker, H., and N. Frenkel.** 1979. *BamI*, *KpnI* and *SalI* restriction enzyme maps of the DNAs of herpes simplex virus strains Justin and F: occurrence of heterogeneities in defined regions of the viral DNA. J. Virol. **32**:429–441.
9. **Lovett, M., C. C. Kuo, K. Holmes, and S. Falkow.** 1980. Plasmids of the genus *Chlamydia*, p. 1250–1252. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease, vol. 2. American Society for Microbiology, Washington, D.C.
10. **Ripa, K. T., and P.-A. Mårdh.** 1977. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated cells. J. Clin. Microbiol. **6**:328–331.
11. **Sarov, I., and Y. Becker.** 1969. Trachoma agent DNA. J. Mol. Biol. **42**:581–589.
12. **Schachter, J.** 1978. Chlamydial infections. N. Engl. J. Med. **298**:428–435, 490–495, 540–549.
13. **Schachter, J., and C. Dawson.** 1978. Human chlamydial infections. PSG Publishing Co., Littleton, Mass.
14. **Tamura, A.** 1964. Biochemical studies on the mechanism of infection of meningopneumonitis agent. Annu. Rep. Inst. Virus Res. Kyoto Univ. **7**:1–13.
15. **Wang, S. P., and J. T. Grayston.** 1970. Immunological relationship between genital TRIC, lymphogranuloma venereum and related organisms in a new microtiter indirect immunofluorescence test. Am. J. Ophthalmol. **70**:367–374.