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Deoxyribonucleic Acid Heterogeneity Between Human and Murine Strains of *Chlamydia trachomatis*[1]

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We compared the polynucleotide sequence relationships of three strains of *Chlamydia trachomatis* of human origin (MRC-1/G, TW-3, and Lgv), one of murine origin (MoPn), and the MN strain of *C. psittaci*. The four strains of *C. trachomatis* have the same base ratio, about 42.5 moles per cent guanine plus cytosine, which is significantly higher than the base ratio of MN (39.5). Single strands of deoxyribonucleic acid (DNA) fragments of MRC-1/G reassociated with immobilized DNA of TW-3 and Lgv almost as well as with the homologous DNA. The duplexes produced in these reactions were about equally thermostable. On the other hand, reassociations between MRC-1/G and MoPn involved 60 or 30% of the DNA, depending on the stringency of the conditions for reassociation, and the duplexes were thermolabile. MoPn reassociated only to a very small degree with MN. We also compared glucose catabolism of MRC-1/G, MoPn, and MN under several sets of conditions. These tests failed to reveal any qualitative phenotypic differences among the three strains. It can be concluded that, judging by polynucleotide sequence, the three human strains of *C. trachomatis* are closely related but appreciably different from a murine strain.

In a previous study of the similarity in polynucleotide sequence of the deoxyribonucleic acids (DNA) of three strains of *Chlamydia trachomatis* (MRC-1/G, Cal 1, and TE-55) and two strains of *C. psittaci* (MN and 5 BC), it was shown that the two species could be clearly separated. DNA strands derived from different species did not form thermostable duplexes. Conversely, when the strains belonged to the same species, binding was almost as extensive as one obtained between homologous strands of DNA (11). This investigation was undertaken to test further the degree of homogeneity within the species *C. trachomatis*.

MATERIALS AND METHODS

The following strains were used: *C. trachomatis* MRC-1/G, associated with inclusion blennorrhea; TW-3, isolated from a case of trachoma; Lgv, isolated from a case of lymphogranuloma venereum; MoPn, agent of mouse pneumonitis; *C. psittaci* MN, agent of meningopneumonitis. The origin of these strains was described by Gordon and Quan (6). *Rickettsia akari* strain MK (8), obtained from B. L. Elisberg, was used in a control experiment. For the preparation of unlabeled DNA and for suspensions for metabolic studies, the microorganisms were grown in chick embryos and were separated from host components as described previously (16, 17). Labeled DNA was obtained from microorganisms grown in cultures of monkey kidney cells (LLC-MK2) irradiated 4 to 7 days previously with cobalt-60 (4,000 r) and placed in 2-liter roller bottles. A combination of adenine-8-^{14}C and cytidine-2-^{14}C was added to the cultures 6 to 18 hr after infection, and the chlamydiae were harvested when the host cells began to lyse, usually 72 to 90 hr after infection. The chlamydiae were separated from host components by enzymatic digestion and differential centrifugation.

DNA preparations were extracted and tested for duplex formation on 25-mm nitrocellulose filters (Carl Schleicher & Schuell Co., Keene, N.H.) as previously described (9). Labeled DNA was fragmented by shearing in a French press at 10,000 psi and was purified by gel filtration through Sephadex G-50 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) covered with a thin layer of Dowex-50 resin (Bio-Rad Laboratories, Richmond, Calif.). The unlabeled DNA, immobilized on nitrocellulose filters, was incubated with labeled DNA fragments overnight at 67 or 75 C, as indicated, in 3 × SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate, *p*H 7.2) containing 0.02% each of Ficoll (Pharmacia), polyvinylpyrrolidone (Oxford Laboratories, Redwood City,

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Calif.), and bovine albumin [fraction V, Armour Pharmaceutical Co., Chicago, Ill. (4)]. The ratio of labeled DNA fragments to unlabeled DNA was 1:100. The amounts of DNA fragments per specimen were 0.25 to 1.0 μg with a radioactivity of 5,000 to 20,000 counts/min. The larger amounts were used in studies that extended to thermal stability of the DNA duplexes. About 50 to 60% of added label was bound in the homologous reaction. The small amount of radioactivity adhering to membranes coated with DNA derived from the MK2 host cells was subtracted from the total, and the per cent binding in heterologous reactions, in comparison with the homologous reaction (defined as 100%), was calculated accordingly.

The thermal stability of the DNA duplexes formed on membrane filters was studied by thermal chromatography (3) by using SSC/30 as the diluent. The temperature required to elute one half of the radioactivity was designated E_m. Molar per cent guanine plus cytosine (G + C) was calculated from the midpoint of thermal denaturation (T_m) of native DNA dissolved in SSC (13).

Determinations of CO_2 production from ^{14}C-labeled substrates were done as described previously (17). Each test vessel contained 0.2 to 0.4 mg of agent protein, as estimated by the technique of Lowry et al. (12), 2.5 mg of bovine plasma albumin (Pentex Corp., Kankakee, Ill.), 1 μmole of Mg^{2+}, 0.2 μmole of Mn^{2+}, 2 μmoles of glucose-1-^{14}C or glucose-*U*-^{14}C (specific activity, 0.1 and 0.2 μc/μmole, respectively), and other reagents, as indicated, in a total volume of 1.0 ml. The diluent was K 36 (16). Each test set consisted of five flasks, three containing viable chlamydiae, one containing boiled *Neisseria meningitidis* cells, and one containing no microorganisms. The radioactivity obtained in the controls was subtracted from that of the test preparations.

RESULTS

The four strains of *C. trachomatis*, including MoPn, appeared to have identical or very similar base compositions (Table 1). The T_m values are somewhat lower than those reported previously for MRC-1/G and two other strains of *C. trachomatis* (11), but are nevertheless significantly higher than those of the MN strain of *C. psittaci*.

TABLE 1. *Base composition of DNA preparations*

Agent	No. of determinations	Mean T_m[a]	Per cent G + C[b]
C. trachomatis			
Strain MRC-1/G	4	86.7	42.4
Strain TW-3	2	86.6	42.1
Strain Lgv	4	86.8	42.7
Strain MoPn	5	86.6	42.2
C. psittaci			
Strain MN	3	85.5	39.4

[a] Range for *C. trachomatis* was 86.4 to 87.1; for *C. psittaci*, 85.1 to 85.7.

[b] Guanine plus cytosine.

TABLE 2. *Per cent binding of labeled DNA fragments to immobilized DNA*[a]

Source of immobilized DNA	Source of labeled DNA fragments		
	C. trachomatis MRC-1/G		*C. trachomatis* MoPn
	67 C	75 C	(67 C)
C. trachomatis			
Strain MRC-1/G	*100*	*100*	65 (5)
Strain TW-3	96 (4)	95 (2)	
Strain Lgv	95 (4)	97 (1)	
Strain MoPn	63[b] (4)	29 (2)	*100*
C. psittaci			
Strain MN	11 (4)		11 (5)

[a] Data presented are the means. The numbers of determinations are indicated in parentheses.

[b] Standard error is 2.7. The other standard errors are 1.0 or less.

The reactions between the DNA of these strains are presented in Table 2. The three human strains of *C. trachomatis* appeared to have a high degree of similarity in polynucleotide sequence. DNA fragments of MRC-1/G bound to the DNA of TW-3 and Lgv almost as well as to the homologous DNA. This occurred at 67 C and under the more restrictive conditions of 75 C (1, 2, 10). MoPn was clearly differentiated from the other three strains of *C. trachomatis*. Its DNA bound a smaller fraction of the DNA fragments of MRC-1/G than the other strains, and binding at 75 C was much less than at 67 C. Almost identical results were obtained at 67 C in the reciprocal reaction with DNA fragments of MoPn. Binding of either DNA fragment, MRC-1/G or MoPn, to *C. psittaci* was very low.

The stability of the DNA duplexes formed at 67 C is depicted in Fig. 1. The E_m values of the two homologous duplexes, MRC-MRC^{14}C (MRC-1/G strain) and MoPn-MoPn^{14}C, were both about 60 to 60.5 C. These values are very similar to the expected T_m of the native DNA suspended in SSC/30 (2, 10). The TW-3-MRC^{14}C and Lgv-MRC^{14}C duplexes had E_m values about 1 C lower. This difference is of doubtful significance. The E_m values of both duplexes formed by MRC-1/G and MoPn, on the other hand, were about 11 to 13 C lower than the homologous duplexes. The data presented in Fig.

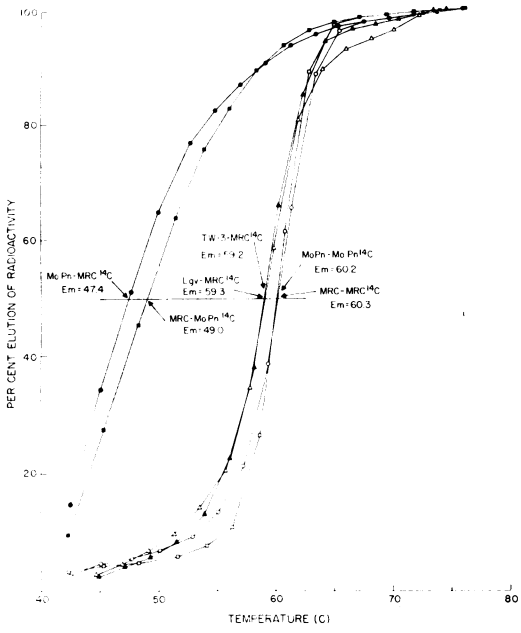

FIG. 1. *Thermal elution patterns of duplexes formed by labeled DNA fragments of strain MRC-1/G (MRC^{14}C) with the DNA of MRC-1/G (MRC), ○; Lgv, ▲; TW-3, △; and MoPn, ●; and by labeled DNA fragments of strain MoPn (MoPn^{14}C) with the DNA of MoPn, □, and MRC-1/G, ■.*

1 further suggest that regions of thermostable binding between these two DNA, if they exist at all, are very small. Thus, it appears that, despite similarity in base composition and moderate capacity for binding, these two DNA differ considerably in polynucleotide sequence.

Since it was previously shown that isolated chlamydial cells display several enzymatic activities (16–19), these reactions were surveyed in an effort to detect phenotypic differences among strains MRC-1/G, MoPn, and MN. As shown in Table 3, with glucose as the substrate, results with the three strains were qualitatively identical. Hexokinase activity was negligible (15), and glucose was metabolized only after it was converted to glucose-6-phosphate by added hexokinase and adenosine triphosphate (ATP). The amount of CO_2 produced without added cofactors was small, and about one third was derived from C_1. The addition of stoichiometric amounts of nicotinamide adenine dinucleotide phosphate (NADP) greatly enhanced CO_2 production from C_1. There is good evidence that, of the two dehydrogenase reactions involving glucose-6-phosphate and 6-phosphogluconate, the second one was rate-limiting. The addition of the first of these two enzymes resulted in reduction of CO_2 production, whereas the addition of the second enzyme was greatly enhancing.

TABLE 3. *Carbon dioxide production from glucose*[a]

Additions[b]	*C. trachomatis*[c]		*C. psittaci* strain MN[c]		*R. akari*	Uninfected yolk sac
	Strain MRC-1/G	Strain MoPn	From yolk sac	From allantoic fluid		
None	0, 0 (0, 1)	0, 0 (0, 2)	0, 0 (0, 0)	0 (0)	0 (0)	
ATP	4, 2	2, 5	3, 2	3	0	
ATP, hexokinase	18, 14 (35, 38)	14, 18 (48, 62)	14, 19 (47, 66)	54 (153)	0 (0)	0 (5)
ATP, hexokinase, NADP	187, 156	119	45, 82	284	1	2
ATP, hexokinase, NADP, glucose-6-phosphate dehydrogenase	123, 69	74	33, 50	89	4	10
ATP, hexokinase, NADP, 6-phosphogluconate dehydrogenase	798, 609	469	287, 321	1,122	12	12

[a] Glucose-*1*-^{14}C (0.1 μc/μmole) or glucose-*U*-^{14}C (0.2 μc/μmole), 0.002 M, was used. The results of individual experiments are shown. They represent CO_2 production from C_1 of glucose, except that the values within parentheses represent total CO_2 production.

[b] Following concentrations were used: ATP, 0.005 M; NADP, 0.002 M; hexokinase, 0.5 micromolar unit/ml; glucose-6-phosphate dehydrogenase, 2 micromolar units/ml; 6-phosphogluconate dehydrogenase, 0.24 micromolar unit/ml. After these experiments were completed, it was observed that with the dehydrogenases sufficient ammonium sulfate was introduced (final concentration 0.03 M) to have a slightly detrimental effect on the activities of the microorganisms.

[c] Values expressed as nanomoles per milligram of protein per 2 hr at 34 C.

There are quantitative differences among the three chlamydial strains, but their significance is not clear. It is interesting to note that the preparation of MN derived from allantoic cavity, on a protein weight basis, was about fourfold more active than the MN preparations obtained from yolk sac. Purification from allantoic fluid is considerably simpler than from yolk sac, does not involve freezing of the microorganisms, and most likely results in less cell injury. Control preparations consisted of yolk sacs, uninfected and infected with *R. akari*, and treated by the procedure used for the chlamydial strains. *R. akari*, like other rickettsiae, does not catabolize glucose (Weiss, *unpublished observations*). The results with the two control preparations suggest that the participation of host enzymes in the reaction with the chlamydiae was negligible.

It was previously shown that MN and the TW-55 strain of *C. trachomatis* catabolized glutamate (17), and MN under certain conditions catabolized succinate, fumarate, and malate (Weiss, *unpublished observations*). These substrates could not be used in comparative studies with yolk sac preparations, because host enzymes were not sufficiently well eliminated.

It can be concluded that the metabolic tests carried out thus far have not revealed clear phenotypic differences among strains of *C. trachomatis* and *C. psittaci*.

DISCUSSION

These results extend previous observations (11) that there is a clear difference in base ratio and polynucleotide sequence between *C. trachomatis* and the MN strain of *C. psittaci*. This sharp distinction may be lost when additional strains are studied (5), but, thus far, MoPn can be placed with the other strains of *C. trachomatis*. This species can be further divided, however, primarily on the basis of the thermal stability of reassociated DNA. (The term "reassociated DNA" refers to heterologous as well as homologous duplexes.) A total of five human strains studied in this and in a previous investigation (11) form thermostable DNA reassociations which involve almost the entire genomes. MoPn and MRC-1/G produce moderately extensive DNA duplexes, but it appears that base pairing is not sufficiently complete for thermostable reassociation.

The correlation between the relative quantity of DNA reassociated in a heterologous reaction and the thermal stability of reassociated DNA has been studied by several investigators (1, 2, 7, 10). In general, when duplex formation obtained under moderately restrictive conditions (67 C in 3× SSC or 60 C in 0.12 M phosphate buffer) is at least 80% of the homologous reaction, similar results are obtained under more restrictive conditions (75 C with either salt solution), and reassociated DNA is thermostable. Conversely, when duplex formation at 67 C is <60%, the amount is even smaller at 75 C, and only a portion of this reassociated DNA, if any, is thermostable. The MRC-MoPn reassociations, despite the fact that they involve 60% of the DNA when elicited at 67 C and 30% at 75 C, appear to be almost entirely thermolabile. These results suggest that, if MRC-1/G and MoPn have a common ancestor, survival of one or both of these strains in a new host did not depend on the conservation of a significant portion of the original genome.

The metabolic tests illustrated in Table 3, which indicate considerable phenotypic similarity among MRC-1/G, MoPn, and MN, are not good indicators of their genotypic differences. It can be similarly implied that the differences in the diseases of trachoma, inclusion blennorrhea, and lymphogranuloma venereum do not reflect the underlying genetic similarity of TW-3, MRC-1/G, and Lgv. It is quite possible that, within certain limits, host and environmental factors, rather than the genetic potential of the infecting chlamydial agent, determine the nature of the disease elicited. This view is supported by the evidence presented by Schachter and Meyer (14) that lymphogranuloma venereum can be associated with either *C. trachomatis* or *C. psittaci*.

It is not practical that a new species of *Chlamydia* be proposed on the basis of our results. Such a proposal can be more appropriately made after it has been demonstrated that several strains resemble MoPn in polynucleotide sequence and that these strains can be differentiated from the other strains of *C. trachomatis* by relatively simple tests of phenotypic characterization.

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