Chromosomal Aberrations Induced by T Strain Mycoplasmas

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Classic mycoplasmas have been reported to induce chromosome changes in leucocyte, amnion, and WI 38 cell cultures. Aula and Nichols (1967) found that *M. salivarium* increased chromosome breakage in human leucocyte cultures; *M. arthritidis* and *M. fermentans* failed to produce chromosome changes, but all three species caused mitotic inhibition. The depletion of arginine from the medium was suggested by these investigators to be responsible for the chromosome changes. Fogh and Fogh (1965) observed a reduction in chromosomes and also an increase in chromosomal aberrations in FL human amnion cells infected with mycoplasmas. The species of mycoplasma was not identified. They observed chromatid breaks within the first week of infection, other changes developed more slowly in infected cell cultures, and included endoreduplications, translocations, dicentric chromosomes, and acentric fragments. New and persistent chromosome varieties were found; these were telocentric, metacentric, and subtelo-centric types. Paton, Jacobs, and Perkins (1967) studying chromosomes in WI 38 cells, reported that three human mycoplasma species caused changes which included chromatid breaks, structural abnormalities, and increased polyplody. *M. pharyngis* (orale 1), *M. fermentans*, and *M. hominis* were studied. Though all produced changes, *M. hominis* and *M. fermentans* were less damaging than *M. pharyngis*. Standbridge *et al* (1969) have found *M. laidlawii*, *M. hyorhinis*, and *M. pulmonis* capable of inducing karyological changes in WI 38 cell cultures.

It, therefore, seemed important and relevant to determine whether the T strain mycoplasmas isolated from human sources could similarly affect the karyology of cells.

**Methods**

Two recent T strain isolates were used for inoculating cultures of peripheral leucocytes. The Boston T strain isolated from the membranes of a middle trimester abortion (Kundsin, Driscoll, and Ming, 1967) and a strain isolated from the cervix of a patient with primary infertility. They were identified as T strains by their small colony size on Shepard's low pH medium (1967), and by the possession of a urease system (Shepard, 1967). Before use in the experiment organisms were grown for 18 to 20 hours in a U9 broth (Shepard, 1967), and 1 ml of broth was used as the inoculum in each flask. Only cultures which developed a change in the pH of the medium, indicating a titre of at least 10⁶ colony-forming units, were used.

Cell cultures were prepared from lymphocytes of clinically normal subjects with no unusual family history for birth defects. 100 ml of medium 199 supplemented with 15 ml of fetal calf serum, 100,000 units of penicillin, and 100,000 units of streptomycin was prepared. This basic medium with supplements was distributed into flasks in 10 ml amounts. To each flask were added 0-8 ml freshly drawn heparinized whole blood and 0-2 ml phytohemagglutinin. 1 ml of an actively growing T strain culture was added. The flasks were incubated on their sides for 4 or 5 days at 36° C. At the end of this period of incubation 1 ml of colchicine (5 × 10⁻⁶M solution) was added to each flask and the flasks incubated 3 more hours before final processing. The cells were suspended by shaking, aliquots were removed for subculture, and the remainder centrifuged. The supernatant was removed. The residual of cells was mixed with 0-075 M KCl up to 10 ml, incubated 15 minutes, again spun down, and the supernatant removed. Fresh fixative made up of three parts methanol to 1 part glacial acetic acid was gradually added up to 10 ml, mixed, and spun. This process was repeated until a clear supernatant was obtained. Tubes were stoppered and placed in a freezer for at least half an hour. Slides were prepared and dried by exposure to an infrared bulb, stained with Giemsa stain and examined. All slides were coded and examined in a single blind manner. Well-spread metaphases were chosen under low power (×160) and examined carefully using oil immersion (×1600). Each chromosome of the metaphase was scanned for integrity and for morphology. No attempt was made to determine the incidence of aneuploidy or the mitotic index, though growth was excellent in all flasks.

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TABLE

CHROMOSOMAL ABERRATIONS INDUCED BY T STRAIN MYCOPLASMAS

<table>
<thead>
<tr>
<th></th>
<th>No. Metaphases Examined</th>
<th>Chromatid Gaps</th>
<th>Isochromatid Gaps</th>
<th>Chromatid Breaks</th>
<th>Isochromatid Breaks</th>
<th>Dicentric Chromosomes</th>
<th>Acentric Fragments</th>
<th>Translocations</th>
<th>Tetraploids</th>
<th>Endoreduplications</th>
<th>Total Errors</th>
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</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RK control</td>
<td>1200</td>
<td>8</td>
<td>31</td>
<td>2</td>
<td>23</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>11</td>
<td>8</td>
<td>26 (2.2%)</td>
</tr>
<tr>
<td>Boston T</td>
<td>1200</td>
<td>16</td>
<td>1</td>
<td>72</td>
<td>4</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>15</td>
<td>41 (3.4%)</td>
</tr>
<tr>
<td>Spitzer</td>
<td>1200</td>
<td>15</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>26 (2.2%)</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LC control</td>
<td>1824</td>
<td>16</td>
<td>8</td>
<td>1</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>71</td>
<td>26 (7.3%)</td>
</tr>
<tr>
<td>Spitzer</td>
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<td>8</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>26</td>
<td>113</td>
<td>144 (12.4%)</td>
</tr>
</tbody>
</table>

* 1 octoploid included.

The chromosome damage was classified as follows: a chromatid gap was defined as a significant segment of chromatid length which stained poorly; the distal segment was not displaced from the axis of the chromatid, however. Isochromatid gaps involve both chromatids at the same point. Since it is impossible in many cases to distinguish between pathological isochromatid gaps and normal secondary constrictions, all were included, and differences were sought between test and control groups. A single chromatid break was defined as a discontinuity in which the distal segment was displaced from the axis of the proximal; isochromatid breaks were similar except that both chromatids were involved at the same point. Translocations and dicentrics were rated separately, as were acentric fragments. Tetraploids and endoreduplications were recorded as encountered on the slides.

The number of cells studied was at least 1,100 in each experiment for test and for control groups.

**Results**

The subcultures taken from each flask following incubation indicated that viable T strains were present in all cases. The Table shows the results of two experiments using two strains.

The difference between total chromosomal errors in leucocyte cultures inoculated with the Boston T and the control was statistically significant (p < 0.001) in experiments I and II. The difference between the test Spitzer strain and control for total errors was also significant at the p < 0.001 level in experiment II. The statistical significance in experiment I (with Boston T strain) was due to the larger frequency of breaks and gaps, while in experiment II it was related to the increase in polyploidy. The test cells inoculated with the Spitzer strain in experiment I induced a slightly higher percentage of errors when compared to controls; the difference was of borderline significance.

**Discussion**

It is noteworthy that the Boston T strain appeared to cause direct damage to the chromosomes, including gaps, breaks, or rearrangements, while the Spitzer strain appeared to result in imperfect separation of chromosomes after division was completed, leading to endoreduplication and tetraploidy. These differences suggest that different T strains may have different modes of action. These experiments indicate that T strains in cell cultures are able to cause cytopathic effects and changes in genetic composition of mammalian cells. Mycoplasmas with characteristics of the large colony size classic strains have been reported as contaminants of many types of cell cultures. It is conceivable that T strains may also be present, particularly in amnion cells, and may be completely missed because the medium used for routine mycoplasma isolation contains thallium acetate and would inhibit the growth of T strains especially on primary isolation. Isolations of T strains from amnion of spontaneous abortions and premature birth have been made repeatedly (Kundsin et al, 1967) and are more frequent than isolations of M. hominis. Since amnion cell cultures may be naturally infected with either or both strains, screening of cells must be done for both types.

Preliminary studies with four other subjects have yielded significant differences between control and infected cells in one of the four. It is known that the presence of certain antibodies and antibiotics can inhibit the growth of T strains; this phenomenon is the basis of the metabolic inhibition test for T strains. It is not unexpected, therefore, that all subjects do not react in terms of chromosome damage to the organism. Conversely, some sera actually enhance growth of T strains as seen in
metabolic inhibition testing. This finding may have some significance in explaining infection with T strains in some individuals and not others.

The cytopathogenetic effects in vitro on lymphocytes of these T strain isolates make speculation on effects in vivo tempting, particularly among spontaneous abortions in humans, where a 20 to 25% incidence of chromosome anomalies has been reported (Carr, 1967).

The method of inducing chromosome aberrations needs further investigation. The investigators who reported cytopathogenetic effects as correlated with arginine deficiency in vitro found this true of only one species, M. salivarium. Arginine deiminase

Fig. 1. Chromosome gaps and breaks induced by Boston T strain. A: single chromatid gap; B: isochromatid gaps (or accentuated secondary constrictions); C: single chromatid break; D: isochromatid break.
was reported to be present in this strain and in two others but did not induce chromosome changes in the other two under the same conditions of experimentation. Woodson, McCarty, and Shepard (1965) compared the amino acid metabolism of three mycoplasma strains and found that two classic strains, *M. hominis* and *M. pharyngis*, utilized arginine with a production of ornithine and ammonia while the T strain they tested did not utilize arginine. It is now known that the T strain does not possess an arginine deiminase and thus cannot alter ornithine concentration of the medium. This suggests strongly that arginine depletion may not be the cause of chromosomal changes with T strain infected cells.

Arginine is only one essential amino acid and it has subsequently been shown in Chinese hamster cells that lack of any one of the other essential amino acids can also result in the same kind of chromosomal abnormalities (Freed and Schatz, 1969).

In the present study, T strains isolated from patients with unsatisfactory reproductive histories...
have been found capable of inducing chromosomal aberrations in small lymphocytes in cultures of peripheral blood. Lack of an essential amino acid other than arginine may explain our finding with T strains.

**Summary**

Two T strains recently isolated from human sources were used to infect lymphocytes from peripheral blood for chromosomal studies. The Boston T strain, isolated from a middle-trimester abortion, and a cervical isolate from a patient with primary infertility were able to induce chromosomal abnormalities in statistically significant numbers. These abnormalities included single and isochromatid gaps, breaks, and tetraploidy.

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**References**

FIG. 4. Polyploidy induced by Boston T strain. A: tetraploidy; B: endoreduplication.
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