Binding, Ingestion, and Multiplication of \textit{Chlamydia trachomatis} (L$_2$ Serovar) in Human Leukocyte Cell Lines

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Chlamydiae are obligate intracellular bacteria that can infect fibroblast and epithelial cell lines in vitro (8) and also enter granulocytes and macrophages (17; K. Register, P. Wyrick, W. Shaber, and J. K. Spitznagel, Abstr. Annu. Meet. Am. Soc. Microbiol., D6, p. 52). Binding of chlamydiae at 37°C by these cells leads directly to bacterial uptake, with ensuing growth, latency, or destruction (10).

We recently demonstrated that the L$_2$ strain (lymphogranuloma venereum [LVG] biovar) of \textit{Chlamydia trachomatis} will bind to but not enter a subpopulation (Ca. 50%) of human peripheral blood B lymphocytes (J. Bard and D. Levitt, Clin. Immunol. Immunopathol., in press). To develop a system for evaluating different types of chlamydial-mammalian cell interactions, we analyzed binding to, entrance of, and growth of L$_2$ serovar in several human leukocyte cell lines and compared these interactions with those of a prototype fibroblast cell line (McCoy). Our results demonstrate four distinct types of chlamydia-host cell interactions at 37°C: (i) minimal to no bacterial binding, (ii) bacterial binding, followed by ingestion and high-level multiplication, (iii) bacterial binding, followed by ingestion but minimal multiplication, and (iv) bacterial binding, but minimal entrance or replication.

\textit{Chlamydia trachomatis} (L$_2$ serovar; L$_2$/434/Bu) was isolated in McCoy cells and purified on Renografin gradients (9). Cell lines were grown in RPMI 1640 containing 10% fetal bovine serum, 2 mM L-glutamine, and gentamicin (10 \mu g/ml) (complete media) and were screened monthly for mycoplasma contamination (7). All cell lines were cloned in soft agar within 3 months of initiation.

Binding and ingestion of L$_2$ were assessed by an immunofluorescence assay (Bard and Levitt, in press) using fluorescein-conjugated mouse monoclonal antibody specific for the L$_2$ serovar (kindly provided by M. Tam, Genetic Systems, Seattle, Wash. [15]) and an irrelevant fluorescein-conjugated mouse immunoglobulin G, MOPC21, as control. Positive cells were evaluated using a Nikon Optiphot fluorescence microscope equipped with epi-illumination; 1,000 to 1,500 cells were counted on each slide.

To monitor the ingestion of chlamydiae, cells were incubated with L$_2$ for 3 to 4 h in complete medium to permit the uptake of surface-bound bacteria. Cells were then treated with pronase (4 mg/ml in Hanks balanced salt solution) for 45 min at 37°C to remove surface-bound chlamydiae from cells. (4-6) or a protease-sensitive molecule recognized by the monoclonal antibody from the bacteria (15; unpublished observations). Only extracellular bacteria were affected by this treatment. Cells were then stained with the fluorescein-conjugated mouse anti-L$_2$ antibody.

To investigate the growth of bacteria within each cell line, chlamydiae were centrifuged onto 2 x 10$^6$ cells (multiplicity of infection [10:1] previously determined to be optimum for four of the cell lines tested), washed, and then resuspended in complete medium containing cycloheximide (1 \mu g/ml). Samples were incubated for either 32 or 48 to 72 h and examined for inclusion bodies (indicative of bacterial growth) or infectious progeny, respectively. The percentage of cells with inclusion bodies was determined by immunofluorescence using fluorescein-conjugated mouse anti-L$_2$ antibody. The yield of infectious \textit{C. trachomatis} was assessed by titration in McCoy cells (2, 13) and represented as inclusion-forming units per milliliter of culture supernatant according to the method of Rothermel et al. (13).

Four patterns of interaction between the leukocyte cell lines and L$_2$ were apparent (Table 1). Two cell lines possessed fewer than 5% of cells binding chlamydiae (group I, Table 1). Six cell lines exhibited significant chlamydial binding (20 to 90% of the cells bound L$_2$), although minimal entrance or growth of bacteria within cells occurred (group II, Table 1). Two cell lines demonstrated sizeable populations of cells which chlamydia bound to and entered, yet bacterial proliferation was hindered (as evidenced by reduced percentages of inclusion-containing cells compared with significant numbers [>20%] of ingesting cells (group III, Table 1). One cell line (Rienta) possessed a large population of cells (50%) which bound (Fig. 1), ingested, and permitted productive multiplication of L$_2$ (group IV, Table 1; Fig. 1). The prototype cell line (McCoy) exhibited the greatest degree of binding, ingestion, and multiplication of bacteria (group IV, Table 1).

Chlamydial attachment appeared to be specific for each cell line for three reasons: (i) different percentages (0.5 to 90%) of cells from the 11 leukocyte cell lines analyzed here bound L$_2$ in a reproducible fashion, (ii) digestion of cell surface proteins with protease (4 mg/ml, 30 min, 37°C) reduced chlamydial binding 4- to 10-fold by some cell lines (Nalm 6, Rienta, McCoy) but did not affect binding by other cell lines (F4, Daudi), (iii) centrifuging bacteria with cells

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(12) improved binding significantly only in 40% of the cell lines.

Despite their clonal derivation, only a fraction of cells from each leukocyte line could bind L2. Binding may correlate with cell cycle status, expression of specific chlamydia-binding molecules, or receptor metabolism. We are currently analyzing these questions using fluorescence-activated cell sorting of chlamydia-binding, synchronized cell populations.

Previous studies have assessed interactions between chlamydiae and cell lines which are believed to be representative of normal hosts (epithelial cells, fibroblasts) or "professional" phagocytes (macrophages, granulocytes) Register et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984; 1, 9, 14, 16-18. Although certain cell lines were only minimally infected by chlamydiae (8), it was not established whether diminished binding, entrance, or growth was the prime factor in the altered host-bacteria interactions. Most studies that have explored binding of LGV to fibroblast or epithelial cell lines have employed mass analyses of radiolabeled bacteria and have not assessed specific interactions on an individual cell basis.

Our results clearly demonstrate that the L2 serovar can bind to cells at physiological temperatures without entering

**TABLE 1. Binding, ingestion, and multiplication of *C. trachomatis* (L2 serovar) in human leukocyte cell lines**

<table>
<thead>
<tr>
<th>Group and cell line</th>
<th>Phenotype*</th>
<th>% Cells binding L2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Cells ingesting L2&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% Inclusion-containing cells&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Yield of progeny&lt;sup&gt;e&lt;/sup&gt; (IFU/ml × 10&lt;sup&gt;3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>HL-60</td>
<td>Promyeloid</td>
<td>0.5 ± 0.2</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.3 ± 0.3</td>
<td>9.8 ± 3.5</td>
</tr>
<tr>
<td>LBW-4</td>
<td>B blast</td>
<td>2 ± 1</td>
<td>ND</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>B lymphocyte</td>
<td>54 ± 7</td>
<td>7 ± 4</td>
<td>&lt;0.1</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Daudi</td>
<td>B lymphocyte</td>
<td>29 ± 5</td>
<td>0.2 ± 0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
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<td>Hurwitz</td>
<td>Plasmablast</td>
<td>23 ± 6</td>
<td>2 ± 0.3</td>
<td>3.2 ± 0.9</td>
<td>8.2 ± 4.6</td>
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<tr>
<td>LR-2</td>
<td>Plasmablast</td>
<td>45 ± 3</td>
<td>8 ± 3</td>
<td>3.3 ± 0.9</td>
<td>13.0 ± 4.4</td>
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<tr>
<td>Molt-3</td>
<td>T blast</td>
<td>90 ± 1</td>
<td>&lt;0.1</td>
<td>0.3 ± 0.2</td>
<td>1.6 ± 0.4</td>
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<tr>
<td>LBW-8</td>
<td>T blast</td>
<td>43 ± 1</td>
<td>&lt;0.1</td>
<td>0.8 ± 0.3</td>
<td>8.1 ± 1.5</td>
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<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalm 6</td>
<td>Pre B</td>
<td>38 ± 1</td>
<td>23 ± 4</td>
<td>6.0 ± 2.1</td>
<td>220 ± 44</td>
</tr>
<tr>
<td>LR-3</td>
<td>Null</td>
<td>45 ± 6</td>
<td>45 ± 3</td>
<td>12.6 ± 3.4</td>
<td>120 ± 54</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Rienta</td>
<td>Null</td>
<td>52 ± 7</td>
<td>ND</td>
<td>46.7 ± 1.5</td>
<td>5,200 ± 33</td>
</tr>
<tr>
<td>McCoy</td>
<td>Fibroblast</td>
<td>&gt;95</td>
<td>92 ± 2</td>
<td>93.0 ± 4.0</td>
<td>19,000 ± 660</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by immunofluorescent and Wright-Giemsa staining.

<sup>b</sup> *C. trachomatis* was incubated with cell lines (10<sup>6</sup>) as described in the text. The percent cells binding L<sub>2</sub> serovar was determined by counting cells positively stained with fluorescein-conjugated mouse monoclonal antibody against L<sub>2</sub> serovar. This antibody did not react with cells which had not been incubated with chlamydiae. Similarly, cells incubated with an irrelevant fluorescein-conjugated antibody (MOPC-21) did not stain (data not shown).

<sup>c</sup> Percentages of cells ingesting L<sub>2</sub> serovar and containing inclusions (indicating bacterial growth) were assessed by immunofluorescence after protease treatment as described in the text. Values represent means ± standard error of the mean from at least three separate experiments.

<sup>d</sup> Yield of infectious progeny was titrated with McCoy cells for 48 to 72 h postinoculation as described in the text. Values represent mean inclusion-forming units per milliliter of supernatant (duplicate samples) ± standard error of the mean from at least three separate experiments, as determined by the method of Rothermel et al. (13).

<sup>e</sup> ND. Not done.

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**FIG. 1.** Binding to and growth of *C. trachomatis* in the Rienta (null) leukocyte cell line. One million Rienta cells were incubated with L<sub>2</sub> for 1 h. Binding was assessed by staining cells with fluorescein-conjugated anti-L<sub>2</sub> after the 1-h incubation period and, inclusions were determined by staining with the same reagent after 32 h of incubation. (A) Phase-contrast micrograph of Rienta cells 1 h postincubation with L<sub>2</sub>. (B) Same field as in panel A stained with anti-L<sub>2</sub>. Arrows in panels A and B indicate corresponding chlamydia-binding cells. Highly fluorescent staining represents aggregates of bacteria. (C) Phase-contrast micrograph of infected Rienta cells 32 h postinfection. (D) Same field as in panel C stained with anti-L<sub>2</sub>. Arrows in panel C indicate representative inclusion-containing cells. Magnification, ×500.
them. This situation also occurs with normal human peripheral blood B lymphocytes (Bard and Levitt, in press), which are activated after exposure to L2 (2). However, there is no clear correlation between a cell line's "phenotype" and its ability to bind, ingest, or permit proliferation of LGV. These cell lines do not seem to be precise correlates of specific cell types, but instead may represent certain stages of leukocyte maturation. Such lines do demonstrate that a wide array of interactions can occur between LGV and different leukocyte (nonadherent) cell lines even in the absence of exogenous substances or altered growth conditions (3, 11, 13).

Finally, analysis of chlamydial interactions with leukocyte cell lines has provided two new pieces of information. (i) Even under normal in vitro growth conditions, LGV can bind avidly to a cell without entering it. (ii) LGV can enter nonphagocytic cell types but not grow. Although we do not know how these cells prevent the entrance of chlamydiae or modify their growth, they provide excellent model systems with which to examine these issues. We are currently studying interactions between B-strain chlamydiae and leukocyte cell lines and separating chlamydia-binding subpopulations with a fluorescence-activated cell sorter.

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LITERATURE CITED