Lymphogranuloma Venereum

II. Characterization of Some Recently Isolated Strains

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Five Bedsonia (Chlamydia) isolates from lymphogranuloma venereum (LGV) patients were tested for inclusion type, sulfonamide sensitivity, and mouse virulence. Two matched the classical description of LGV agents. Two were not virulent for mice by the intracerebral route, therefore fitting the description for trachoma-inclusion conjunctivitis agents. One was highly virulent for mice and sulfonamide-resistant, and produced inclusions that did not stain with iodine, all characteristics generally associated with avian bedsoniae. A sixth isolate could not be adequately tested due to poor infective yields. Because of this variety of properties within the Bedsonia group, the term LGV might more appropriately be reserved for clinical disease rather than to describe a particular bedsonial agent.

A member of the Bedsonia (Chlamydia) group of microorganisms is the causative agent of the venereal disease lymphogranuloma venereum (LGV; 2). The seventh edition of Bergey's Manual of Determinative Bacteriology refers to the agent as Miyagawanella lymphogranulomatosi and describes it as sensitive to sulfonamides, not productive of an iodine-staining inclusion, and virulent for mice when inoculated by the intracerebral (IC) and intranasal (IN) routes but not by the intraperitoneal (IP) route. Many strains of LGV have been isolated (8, 9), but few studies thereof have been reported since the mid-1940's. The only LGV strains extant have a laboratory history of approximately 30 years. In 1967 it was reported that an agent isolated from a patient with clinical LGV had the characteristics of an avian bedsonial strain (sulfa-resistant, highly virulent for mice, and nonproductive of glycogen; 6). This paper presents results of laboratory studies on some bedsonial strains isolated from clinical cases of LGV. Most patients were servicemen and seamen returning from Southeast Asia. Clinical features and results of diagnostic tests for these patients will be published elsewhere (J. Schachter et al., J. Infect. Diseases, in press).

MATERIALS AND METHODS

Isolation. Agents were isolated from bubo pus by techniques described in an earlier paper (7). Yolk sacs (YS) of embryonated hens' eggs were used, but in some instances mice were inoculated IC. Bubo pus and YS suspensions were tested for bacteriological sterility by inoculating blood-agar plates and thio-
Hanks balanced salt solution and incubated at 35°C in the presence of a maintenance medium of Eagle Minimum Essential Medium and 2% serum, supplemented with 0.1% glucose. Cover slips were removed at intervals for staining.

**Staining techniques.** For the iodine stain, cell monolayers on cover slips were fixed in methanol for 5 min, dried, and stained with Lugol solution for 5 min. The Lugol solution was then washed off with tap water and the preparations were examined as wet mounts. The same cover slips were then decolorized in methanol, dried, and stained with Giemsa (3).

A modified Macchiavello technique (3) was used to stain both YS and mouse-organ smears. Dead animals were included in titrations only when smears of their organs contained elementary bodies.

**Sulfadiazine sensitivity.** Isolates were titrated in ovo. The effect of sulfadiazine was measured by inoculating 0.1 ml of sterile distilled water containing 1 mg of sulfadiazine into the YS of eggs immediately after the agent was inoculated. The \(LD_{50}\) values with and without sulfadiazine were determined in parallel.

**Isolates tested.** The isolates used, the passage level tested, and the year of isolation, respectively, were as follows: 33-L, YS-7, 1964; 404-B, YS-4, 1967; 434-B, YS-5, 1967; 440-L, YS-4, 1967; 470-L, YS-4, 1968. Another isolate, 420-L (1967), was also tested, but because of its poor growth in the YS a stable pool could not be prepared.

**RESULTS**

All agents used were previously isolated in the YS of embryonated hens' eggs. Isolation attempts in mice failed. Isolates were identified as Bedsonia by serial passage of characteristic elementary bodies and by demonstrating group antigen in YS. The complement-fixing antigen titers were 1:256 or higher. All isolates except 420-L were regularly lethal for eggs, and large YS pools could be prepared to perform comparative tests on the same material. Lethality was irregular for isolate 420-L, and only occasionally did all eggs in an experiment die at a 50% inoculum, so that a pool could not be prepared. Isolate 33-L was highly virulent for eggs, with an \(LD_{50}\) of 10^4 or greater. The infectious and lethal doses of this isolate were essentially the same; surviving embryos seldom gave positive test results for the agent. Other isolates tested (404, 434, 440, and 470) were moderately virulent for the egg embryo, with \(LD_{50}\) values of about 10^4 to 10^5, and their infectious doses exceeded their lethal doses by approximately 2 log. YS smears and suspensions from surviving embryos were often as heavily infected as those prepared from dead embryos.

Table 1 shows the results of titrations in mice. Isolate 33-L was lethal and had a high titer by all routes tested. Isolates 434 and 440 were lethal, with low titers, when inoculated by the IC and IN routes but were not lethal by the IP route.

**DISCUSSION**

The typical description of the LGV agent, regarding mouse virulence, sulfonamide sensitivity, and inclusion type, is based upon work performed 20 to 30 years ago or on studies of an
TABLE 2. Effect of sulfadiazine on LGV isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No sulfadiazine</th>
<th>Sulfadiazine added (1 mg/embryo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33-L</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>404-B</td>
<td>4.5</td>
<td>X</td>
</tr>
<tr>
<td>434-B</td>
<td>4.2</td>
<td>X</td>
</tr>
<tr>
<td>440-L</td>
<td>5.2</td>
<td>X</td>
</tr>
<tr>
<td>470-L</td>
<td>5.5</td>
<td>X</td>
</tr>
</tbody>
</table>

a $\log_{10}$ egg LD$_{50}$/g of YS.

b $X$ = No deaths at 20% YS suspension.

LGV isolate (strain JH) having a 30-year history of laboratory passage. The present study of six recent low-passage isolates reveals some differences in their properties. All six isolates were derived from patients with classical LGV; five were from bubo aspirates and the sixth was from an excised perirectal lymph node. Isolate 420-L, an extremely feeble grower in YS, did not grow at all in the other systems tested. The characteristics of isolate 33-L were those usually associated with some avian bedsoniae, that is, highly virulent for mice (3), iodine-negative, and sulfonamide-resistant (1, 4). Such agents are not known to be prevalent in the clinical syndrome, but they could provide one explanation for the sporadic failures of sulfonamide treatment. It need not be the only explanation, for tetracycline treatment sometimes also fails, and all these isolates are sensitive to tetracycline.

The characteristics of isolates 434 and 440 are identical to those of the prototype JH strain of LGV [which produces an iodine-staining inclusion (1), in contradistinction to the description given in Bergey's Manual]. Isolates 404 and 470, however, differ from the prototype in mouse virulence. The danger of using pathogenicity patterns for isolate typing is well known, but in the absence of more refined markers, mouse virulence is often used for this purpose. This marker is particularly important in the differential identification of trachoma-inclusion-conjunctivitis (TRIC) agents, bedsoniae which may be sexually transmitted in man. A bedsonia isolated from the human genital tract is generally considered as an LGV agent if it kills mice inoculated by the IC route and as a TRIC agent if it does not. Therefore isolates 404 and 470 would have been considered as TRIC agents had they been derived from the urethra and not from an inguinal bubo. The only other distinctions are the ability of TRIC agents to produce follicular conjunctivitis in primates (LGV agents presumably do not) and the cytotoxic effects and rapid growth seen in tissue culture with these LGV agents. A test in primates would be impractical for routine use with all bedsonial isolates derived from the human genital tract. The pathogenicity for the conjunctiva of these LGV isolates is being studied.

The original attempts at isolating LGV agents in YS resulted in at least one isolate obtained in ovo that could not be maintained in mice (8). The mouse was the most popular indicator host in early studies of LGV. Obviously, agents not lethal for mice could not have been isolated by this method. Studies like that of Wall (9), showing the mouse superior to the egg for isolation, may have reflected the properties of the agents prevalent in a particular area at a specific time. Apparently LGV agents need not be virulent for mice; therefore, a major criterion for differentiating between LGV and TRIC agents is lost. Possibly there are no valid differences, for there may be overlap within the groups if enough isolates are tested.

LGV is a clinical entity, and the term should not be used to refer to a single type of Bedsonia. Possibly any Bedsonia may produce this clinical entity, but certainly the agents isolated from LGV patients in this study present a spectrum of qualities within the Bedsonia group.

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LITERATURE CITED