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Serotyping of *Chlamydia*: Antibodies to Lymphogranuloma Venereum Strains Compared by Microimmunofluorescence and Neutralization Tests

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The microimmunofluorescence test with hyperimmune rooster sera against lymphogranuloma venereum strains gave the same serotype patterns obtained in the standard mouse serum system. Results in a plaque reduction test appeared similar when only 3 prototype strains were tested, but differed when a battery of 11 strains were tested.

Chlamydiae possess a number of different antigens. The group antigen common to all chlamydiae and measurable by complement fixation tests is routinely used to diagnose psittacosis and lymphogranuloma venereum (LGV). Specific antigens are more difficult to demonstrate. By using the microimmunofluorescence (micro-IF) test, Wang and Grayston have found a number of specific antigenic patterns within *Chlamydia trachomatis* strains (2, 7). They have identified 12 serotypes of trachoma-inclusion conjunctivitis agents (designated A through K) and 3 serotypes of lymphogranuloma venereum isolates (designated L-1, L-2, and L-3). Specific antigens of *C. psittaci* have been shown to reside in cell walls, and specific reactions are demonstrable by the plaque reduction test (PRT) (1, 3). The plaque reduction assay is not generally applicable to *C. trachomatis* strains because most of them do not grow well enough in cell culture to produce plaques. However, because LGV isolates (within *C. trachomatis*) are cytopathogenic and will produce plaques, they were used to compare plaque-reducing antibody patterns to micro-IF antibody patterns in the same anti-LGV sera.

The immunization schedules of the White Leghorn roosters used to produce antisera have been published (1). In general, these roosters require between 9 and 18 inoculations of yolk sac-grown agent—considerable antigenic stimulation—before they will produce neutralizing antibodies. The micro-IF test was performed by previously published techniques, using serial dilutions of antisera against an array of standardized antigen preparations placed in a template pattern on slides (7). Fluorescein-conjugated anti-chicken globulin was obtained from Antibodies, Inc. The plaque reduction test was performed as described by Banks et al. (1).

The eight LGV strains, designated by numbers, were isolated in San Francisco by the yolk sac method from aspirates of bubos of patients with typical LGV, except for 678, which was obtained from blood. Clinical details of some of these patients have been published (6). The JH strain is a standard laboratory LGV strain. The T'ang and LB-1 strains are representative of the so-called fast trachoma-inclusion conjunctivitis agents and have been identified as LGV serotype L-2 (8).

The micro-IF assays showed that the LGV strains were closely related and had marked cross-reactions (Table 1). However, these agents, previously typed by the standard micro-IF test using mouse antiserum, fell into the same general pattern of reactivity and yielded the same groupings with rooster antiserum. The rooster antisera were high titered, ranging from a low of 1:128 to a high of 1:8,192. They did not cross-react with trachoma-inclusion conjunctivitis immunotypes A and C and showed low-grade cross-reactions to B, D, and E (not shown in Table 1). Although the serotyping results were not quite as clear-cut as some obtained with early mouse sera, the patterns were virtually identical.

We had difficulty producing neutralizing antisera for the PRT. Despite the fact that four roosters were immunized with each strain, we failed to produce neutralizing antisera to some of the LGV isolates. For this reason, results presented for the micro-IF test contain more antisera than could be tested with the plaque reduction test. The results of plaque reduction tests are shown in Table 2. The neutralizing antibody levels were generally much lower than those obtained in the micro-IF test; only two of the eight antisera yielded homologous titers greater than 1:100.

TABLE 1. *Micro-IF test with rooster antisera against LGV isolates*

Antiserum	% of homologous titer[a] with antigen: L-1 440	L-1 810	L-2 434	L-2 470	L-2 526	L-2 678	L-2 JH	L-2 T'ang	L-3 (404)
440 (8,192)[b]	100	100	6	13	25	25	25	6	13
810 (4,096)	200	100	13	25	25	25	13	13	13
434 (1,024)	25	25	100	100	100	100	50	50	6
470 (2,048)	25	50	100	100	100	50	50	25	13
514 (128)	0	0	100	50	100	50	50	25	0
526 (2,048)	50	50	100	50	100	100	100	50	0
678 (8,192)	25	25	ND[c]	50	100	100	100	50	50
JH (512)	50	13	100	100	100	200	100	50	13
T'ang (2,048)	50	50	100	100	100	100	100	100	50
LB-1 (4,096)	13	13	100	100	100	50	50	25	13
404 (512)	13	13	13	6	13	13	25	13	100

[a] Groups are boxed on the basis of antigenic types determined by standard typing test with mouse sera (note good agreement with this typing procedure).
[b] Homologous titer.
[c] ND, Not done.

TABLE 2. *Plaque reduction test with rooster antisera against LGV isolates*

Antiserum	% of homologous titer[a] with antigen: L-1 440	L-1 810	L-2 434	L-2 470	L-2 514	L-2 526	L-2 678	L-2 JH	L-2 T'ang	L-2 LB-1	L-3 (404)
440 (447)[b]	100	33	12	16	36	5	9	7	<2	<2	36
434 (143)	11	21	100	22	80	48	28	22	<7	<7	11
470 (17)	100	100	—	100	—	100	200	200	—	—	—
514 (32)	—	—	—	—	100	—	—	—	—	—	—
526 (17)	—	—	—	—	100	100	—	—	—	—	—
678 (46)	—	57	50	33	—	25	100	33	35	50	35
LB-1 (17)	250	150	—	—	—	600	250	350	60	100	—
404 (76)	28	—	33	33	—	—	—	—	—	—	100

[a] —, No neutralization observed at lowest dilution (1:10); groups are boxed on the basis of antigenic types determined by standard mouse typing system (note poor fit).
[b] Homologous titers.

When PRT results for only the prototype strains of L-1 (440), L-2 (434), and L-3 (404) are considered, the extent of cross-reaction was generally similar to that obtained by the micro-IF test. However, when additional strains were tested, the PRT antibody patterns did not parallel those obtained with the micro-IF test (Table 2). Indeed, when the PRT results were used, it was difficult to discern specific serotypes at all. For example, isolate 514, which is a typical L-2 in the micro-IF test, appears to be antigenically unique in the PRT. The L-3 isolate, 404, is clearly different from the other isolates by the micro-IF test and would have been so identified by PRT. However, the considerable antigenic heterogeneity demonstrated by PRT within the micro-IF serotype L-2 is in marked contrast to the homogeneous results obtained in the micro-IF test. In a few instances, most striking with the serum against LB-1, heterologous reactions were much higher than the homologous reactions.

The type L-1 440 antiserum did appear to yield PRT results generally analogous to those obtained with the same serum in the micro-IF test; however, in the two-way crosses the 440 antigen showed much more cross-reactivity with some type L-2 antisera.

Perhaps the PRT and micro-IF test results do not parallel each other because the PRT test is less sensitive or because there are more variables inherent in the performance of this test. The generally lower titers observed in the PRT may account for some of the discrepancies. However, it is also possible that the two tests measure different antigen-antibody interactions. Pre-

vious studies have shown that complement-fixing antibodies and neutralizing antibodies are not the same and that complement-fixing antibodies and micro-IF antibodies differ (1, 4). With some sera of relatively high neutralizing antibody capacity, marked differences within a group that was homogeneous by the micro-IF test could be observed (for example, 434 antiserum). The two tests possibly measure different antibodies, and the PRT neutralizing antibodies react with different surface antigens from those detected in the micro-IF test.

A heartening result of this study, from a purely technical viewpoint, was that high titers and consistent patterns were observed in the micro-IF test with hyperimmune rooster antisera. Type-specific antibody responses have been noted to *C. trachomatis* infections in humans and subhuman primates and in mice bled shortly after the second of two inoculations. The fact that hyperimmunization did not obliterate the type-specific response and that the rooster antibody titers were so high suggests the possibility that alternate animal sources may be useful in producing antisera for typing chlamydiae by the micro-IF test. Such antisera would facilitate application of the micro-IF test which currently employs antisera produced in mice, a host whose serum yield is small.

It is clear that the micro-IF test is the test of choice for typing these *C. trachomatis* strains. This test certainly deserves further study in the typing of *C. psittaci* strains, in which only very broad grouping is obtained by plaque reduction assay (5).

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