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Development of a Monoclonal Antibody to a *Ureaplasma urealyticum* Serotype 9 Antigen

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We produced a monoclonal antibody (MAb) to *Ureaplasma urealyticum* Vancouver, the serotype 9 standard strain. By immunoblotting, this MAb showed a single, 85-kDa band with the homologous serotype and a minor, 100-kDa band with serotype 2 but did not react with any other serotype standard strain. Clinical isolates of *U. urealyticum* were tested with this MAb and with two sets of polyclonal antisera against the 14 serotype standard strains. The use of MAb 9-2H9 correctly identified certain serotype 9 strains but did not react with wild-type strains lacking the serotype 9 determinant.

The establishment of *Ureaplasma urealyticum* as a member of the normal genital flora has made its role in genitourinary tract disease difficult to define. Human inoculation studies finally demonstrated the pathogenicity of this organism in some cases of nongonococcal urethritis in males (22). The results of certain prospective studies (2, 3, 8, 11) and case reports (1, 7) have suggested a role for *U. urealyticum* in some infections of the female genital tract. The fact that many pregnant women are colonized with ureaplasmas but that few of these infections are associated with adverse effects has raised the possibility of differential host susceptibility or strain pathogenicity. *U. urealyticum* strains comprise two biovars. The parvo biovar is comprised of serotypes 1, 3, 6, and 14, and the T960 biovar is comprised of serotypes 2, 4, 5, 7, 8, 9, 10, 11, 12, and 13 (17). An association between serotype and/or biovar and pathogenicity has been hypothesized. Reliable means of determining the biovar can be achieved by PCR (18), but the division of the biovars by serotyping is beset with problems (21). Some of the relatively few reports have indicated an association between particular serotypes and disease (12, 19), while others have not (9, 13, 16). Current methodologies rely upon nonstandardized reagents, i.e., polyclonal antibodies (PAbs) to whole-cell antigens. The expression of multiple specificities by a single, purified strain of *U. urealyticum* (12, 21) confounds the interpretation of the data. Serotyping with monoclonal antibodies (MAbs) to serotype-specific antigens could reduce this problem. MAbs also could be used to identify the location of these determinants and to assist in their further characterization (4, 5, 24, 26). Antigenic diversity has been identified among isolates of *U. urealyticum* in vitro for serotypes 4 (4) and 3 (5, 24) and in vivo for serotype 3 (25) and may be essential for understanding pathogenicity. For example, specific determinants might allow a strain to escape human immune responses or to express a distinct property that promotes invasiveness.

Serotype-specific MAbs have been described for serotypes 1, 3, 4, 6, 8, and 10 (4, 5, 24). MAbs reacting with more than one serotype have also been described. Cheng et al. described a MAb reacting with serotypes 3 and 14 (5). Thirkell et al. (23) described a MAb which reacts with all 14 serotypes and also, based on immunoblotting patterns, distinguishes the two seroclusters or biovars. Other MAbs have been described by Watson et al. (24); one reacted with all 14 serotypes, while another reacted with all serotypes except types 2 and 5.

We prepared MAbs against the *U. urealyticum* serotype 9 standard strain (strain Vancouver, progenitor of ATCC 33175) (6, 14), for which no serotype-specific antigens had been defined. MAbs were produced as previously described (4). Briefly, BALB/c mice were injected intraperitoneally every 2 weeks with 0.5 ml of antigen (washed whole cells, containing approximately 10^9 color-changing units ml^{-1} of *U. urealyticum* serotype 9 reference strain). Freund's complete adjuvant was added to the first injection; a final booster dose of 0.2 ml of the same antigen preparation was given through tail vein injection 3 days before fusion. Fusion was performed with spleen cells from immunized mice and nonsecreting P3-X63-Ag 8.653 mouse myeloma cells. The hybridoma clones were screened for the production of antibodies by colony epifluorescence (10), with the serotype 9 reference strain used as the antigen.

We note that the serotype 9 standard antigen used as the immunogen was from the same initial source as that used for preparing PAb-C (a set of PAbs produced in Canada) and PAb-B (a set of PAbs produced in Brussels, Belgium). After immunization with *U. urealyticum* serotype 9, one reactive clone, MAb 9-2H9, was identified by colony epifluorescence; it reacted with >90% of the colonies of the serotype 9 standard but not with the remaining 13 serotypes. When immunoblotting was performed (Fig. 1), MAb 9-2H9 reacted strongly with a single band of ~85 kDa with the serotype 9 standard strain but also weakly with a single band of ~100 kDa with the serotype 2 standard strain. It did not react with the other 12 standard strains. Thus, although this MAb was not completely specific for type 9, it should be useful for serotyping by colony epifluorescence.

MAb 9-2H9 was evaluated with seven clinical isolates. Earlier, in colony epifluorescence tests (20), these isolates had demonstrated reactivity with a serotype 9 polyclonal antiserum (16, 18) from PAb-C, the antiserum set prepared in Canada (15). The seven strains were now retested with PAb-B, the set

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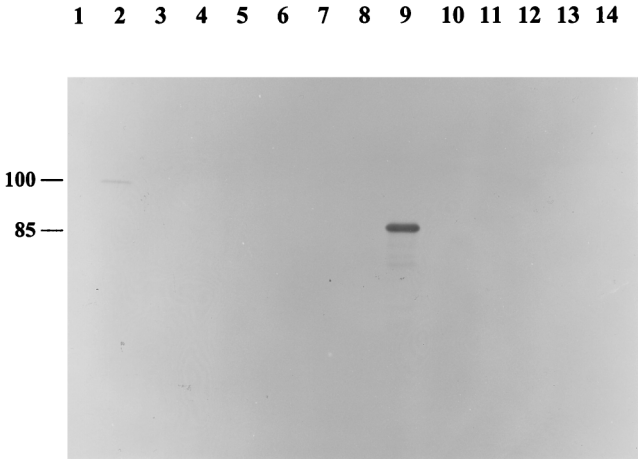

FIG. 1. Immunoblot showing the reaction pattern of MAb 9-2H9 (immunoglobulin G2a, specific to serotypes 2 and 9). Lanes 1 to 14 contain whole-cell preparations of the 14 serotype standard strains of *U. urealyticum*. Molecular masses of the bands, in kilodaltons, are indicated to the left of the gel.

of polyclonal antisera prepared in Belgium (12), and also with MAb 9-2H9 and MAbs to serotypes 1, 3, 4, 6, 7, and 14. DNA from these strains also was tested by 16S rRNA-based, biovar-specific PCRs (18). All results are shown in Table 1.

Of the seven isolates that the PAb-C reagent had recognized as bearing the serotype 9 determinant (as a single specificity or as one of multiple specificities), only four were recognized by PAb-B. The discrepancy between the two anti-serotype 9 antisera could reflect differences in the protocols used for the cultivation and preparation of the immunogen, immunization, or testing. Antibodies to the same antigen could be directed against different epitopes and thus elicit different test results. Only one study has examined the reproducibility of serotyping clinical isolates of *U. urealyticum* with polyclonal antisera (21), and it was limited to a single laboratory using a single set of reagents. Reproducibility of each serotype specificity ranged from 100 down to 67%, with an overall agreement of 87%. The interpretation of data obtained by using such reagents can confuse rather than clarify the objective of the research, which in this instance was the role of strain variation in disease. The present study is the first to compare *U. urealyticum* serotyping results obtained by two laboratories using nonidentical immunoreagents.

TABLE 1. Results of colony epifluorescence tests on selected *U. urealyticum* strains by using PAbs and MAbs

Strain	Serotype based on reactivity to[a]:		Serotype based on MAbs[b]	Biovar
	PAb-C	PAb-B		
JR14	9	9 (10, 11)	9	T960
SH	2, 9, 13	9	9	T960
345	9	9	9	T960
RH 1627	9 (7, 8, 11)	9 (2, 8)	9	T960[c]
1070/77	9, 11, 13	8 (2, 5, 7, 11)	NR[d]	T960
853/78	2, 8, 9, 11	8 (2, 11)	NR	T960
RH 185	2, 5, 7, 9, 14	2, 5	NR	T960[c]

[a] Serotype determinants shown within parentheses gave a weaker reaction than the homologous serotype did.

[b] MAbs were available for the serotype-specific determinants 1, 3, 4, 6, 7, 9, and 14.

[c] Biovar determined on the isolate from a different tissue than that used for serotyping.

[d] NR, not reactive with these MAbs.

Although clinical isolates commonly show polyreactivity with polyclonal antisera (21), polyreactivity clearly reduces the utility of serotyping (21). In epifluorescence tests with either the 13 heterologous serotype standard strains or with 28 sequential isolates (17 of which had been thrice subcloned by limiting dilutions [15] to ensure purity), the partial set of MAbs available to us did not exhibit any polyreactivity (Table 1).

The four strains that reacted with the serotype 9 reagent of both PAb-C and PAb-B also reacted with MAb 9-2H9. The other three strains that reacted with only PAb-C anti-serotype 9 antiserum demonstrated other specificities for which MAbs were not available (Table 1).

When clinical isolates have been typed in Canada or in Belgium, with the full set of 14 specificities, the incidence of serotype 3 has predominated and the incidence of serotype 9 has been in the range of <1 to 15% (12, 16).

In conclusion, we have prepared a MAb to the serotype 9 determinant of *U. urealyticum*. Although this MAb is heterospecific by immunoblotting, it appears to have utility as a reagent for serotyping by colony epifluorescence.

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