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Identification of Serotype 1-, 3-, and 6-Specific Antigens of *Ureaplasma urealyticum* by Using Monoclonal Antibodies

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Little is known about the antigens responsible for serotype specificity in *Ureaplasma urealyticum*. We produced monoclonal antibodies to *U. urealyticum* serotypes 1, 3, and 6, the serotypes most commonly found in pregnant women, and analyzed serotype-specific antigens for the three serotypes. Clinical isolates belonging to serotype 1, 3, or 6 were tested in immunoblots with these monoclonal antibodies. The immunoblot patterns of these isolates were, in most cases, different from each other as well as from those of the reference strains, indicating a high rate of antigenic variation among *U. urealyticum* strains.

Ureaplasma urealyticum is a common inhabitant of the human lower genital tract. It has been implicated in diseases of the genitourinary tract, unfavorable pregnancy outcomes, and recently, infections in newborns (1, 2, 5, 12). Because of the difficulties in establishing the pathogenic role of *U. urealyticum* in diseases of the reproductive tract, it has been postulated that only certain subgroups of *U. urealyticum* are associated with disease. At present, 14 serotypes have been defined (10). Several serotyping studies with polyclonal antisera (polyclonal antibodies [PAbs]) have been reported; those studies used such techniques as the colony indirect immunofluorescence assay (colony-IFA), the growth inhibition test, and the modified metabolic inhibition test. A possible association of certain serotypes with disease was found by some investigators but not by others (4, 9, 11). Serotyping of *U. urealyticum* with PAbs in the colony-IFA is subjected to problems, making it sometimes difficult to interpret the serotyping results. One of the problems encountered is that clinical isolates can express multiple specificities; colonies of *U. urealyticum* react with more than one antiserum, and this is done without clear evidence of mixed serotypes. Another problem encountered is the lack of reproducibility of the serotyping assay. This can be due either to the difficulty of scoring the fluorescence intensity or to the inadequate number of colonies on the agar surface, making it difficult to detect mixed cultures. When serotyping is performed on agar plugs cut from the original agar plate, lack of reproducibility can be due to the inversion of agar plugs during washing and staining. The lack of agreement between different serotyping studies may be due in part to these interpretation problems encountered in serotyping *U. urealyticum* when using PAbs (4, 11). A more objective and standardized serotyping method and better knowledge of the antigens responsible for *U. urealyticum* serotype specificity are needed. Serotype-specific monoclonal antibodies (MAbs) could be one of the tools needed to achieve this objective. Moreover, MAbs may establish the basis of cross-reactions. It is, however, not until recently, with the development of MAbs, that serotype-specific antigens are being identified (3, 13, 14, 16).

In a previous serotyping assay, we studied the incidence of *U. urealyticum* serotypes among different patient populations; in our population, 75% of the *U. urealyticum* isolates belonged to serotype 1, 3, or 6 (4). In the study described here, MAbs against these frequently encountered serotypes were produced, serotype-specific antigens were identified, and their use in serotyping clinical isolates was evaluated in comparison with the use of PAbs.

Reference strains of *U. urealyticum* serotypes 1 to 10 were supplied by E. A. Freund (Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark) and those of serotypes 11 to 14 were supplied by J. A. Robertson (Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada). MAbs were produced as described previously (3).

Two clones secreting MAbs against serotype 1, eight clones secreting MAbs against serotype 3, and three clones secreting MAbs against serotype 6 were produced. They were first screened by colony-IFA (6) with the corresponding serotype reference strains. A strong fluorescence was observed on more than 90% of the respective *U. urealyticum* colonies by all MAbs. The reactivities of these MAbs were subsequently checked by colony-IFA and immunoblotting by using all 14 reference strains. The immunoblotting was performed as described previously (3).

The MAbs against serotype 1 are defined as serotype 1 specific since no cross-reactions with other serotypes were found either in immunofluorescence assays or in immunoblots. In immunoblots, they reacted with a single band with a molecular mass of 83 kDa.

Two of the eight MAbs developed against serotype 3 showed no cross-reaction with the other 13 *U. urealyticum* reference strains in either colony-IFA or immunoblots. These MAbs are referred to as type 3 specific. The two type 3-specific MAbs showed almost the same pattern in immunoblots; two bands with molecular masses of 71 and 72 kDa, respectively, were identified. Weak reactions with the serotype 3 reference strain were also seen with bands larger than 72 kDa. When 20 μg of antigen was loaded, no apparent stronger band could be found, but if the antigen load was doubled, about three more bands were observed in the region. Six of the eight MAbs showed reactions both in the colony-IFA and immunoblots with serotype 3 and 14 strains. These MAbs are referred to as type 3/14 specific. The six type 3/14-specific MAbs reacted in immunoblots with the serotype 3 reference strain in a similar way as the type 3-specific MAbs did. Besides the 71- and 72-kDa bands, these MAbs recognized a clear multiple-band ladder pattern below the 71-kDa band which was not found by type 3-specific MAbs (data not shown). The serotype 3/14-specific MAbs

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TABLE 1. Results of the colony-IFA with MAbs on *U. urealyticum* clinical isolates

Strain	Reaction with PAbs 1 to 14 (serotype)	Reaction with MAbs		
		Type 1 specific	Type 3 specific	Type 6 specific
B67	1	+	−	−
Va51	1/2	−	−	−
Ve32	1/2	−	−	−
B5	3	−	+	−
B53	3	−	+	−
Da21	3	−	+	−
DF18	3	−	+	−
Df24	3	−	+	−
E5	3	−	+	−
E20	3	−	+	−
G17	3	−	+	−
L12	3	−	+	−
P42	3	−	+	−
Ve30	3/10	−	+	−
B43	4	−	−	−
A20	6	−	−	+
B61	6 weak	−	−	+
L28	6	−	−	+
Va46	6/10	−	−	+
E16	6	−	−	−
C19	−	−	−	−

reacted in immunoblots with serotype 14 reference strain with a single band with a molecular mass of 84 kDa. Serotype 3- and 3/14-specific MAbs, showing similar but not identical patterns, have been described previously (14, 16).

The three MAbs developed against serotype 6 showed no cross-reaction with the other 13 *U. urealyticum* reference strains either in colony-IFAs or in immunoblots. In immunoblots, the MAbs reacted with *Ureaplasma* antigens only when they were separated by a 7.5% separating gel and failed to react when they were separated by a 10% separating gel. Most probably, the serotype 6 antigen was unable to enter into the 10% separating gel; since the immunoblot was repeated at least three times, it is likely that this was due to the nature of the antigen and not to an experimental error. The serotype 6-specific MAbs reacted with two bands with molecular masses of 97 and 59 kDa, respectively, in serotype 6 reference strain. MAbs reacting with serotype 6 in immunoblots were also reported by other investigators (13, 14). However, all of these cross-reacted with other serotypes.

To evaluate our MAbs, 21 *U. urealyticum* clinical isolates were cloned three times by a broth dilution method described by Robertson and Stemke (10). After subcloning, the strains were tested in the colony-IFA with polyclonal antisera 1 to 14 and the following MAbs: serotype 1 specific, serotype 3 specific, serotype 3/14 specific, and serotype 6 specific. These strains, which were isolated from the cervix, placenta, and semen, were selected on the basis of their previous serotyping results, in which they showed reactions with polyclonal antiserum 1, 3, or 6 (4). Results for the 21 strains are summarized in Table 1. When testing these clinical isolates with PAbs 1 to 14, 16 strains reacted only with one polyclonal antiserum, as follows: 1 strain reacted with antiserum 1, 10 strains reacted with antiserum 3, 1 strain reacted with antiserum 4, and 4 strains reacted with antiserum 6. Four other strains reacted with two different polyclonal antibodies PAbs, as follows: two strains reacted with PAbs 1 and 2, one strain reacted with PAbs 3 and 10, and one strain reacted with PAbs 6 and 10. One strain was not typeable with the polyclonal antisera. General agreement between results obtained with PAbs and MAbs were good. Comparison of the results for polyclonal antisera 1 to 14 with those obtained with the MAbs revealed that all monoreacting strains reacted with the corresponding MAb except for one type 6 strain, which was not detected by the MAbs. The strain not typeable with PAbs was also not reactive with our MAbs. The strains that reacted with more than one polyclonal antisera showed different results with the MAbs. Since they were subcloned, no mixed serotypes were expected; these dual reactions in polyclonal antisera could be explained by the cross-reactivities between the two polyclonal antibodies for these strains. The strain that reacted with PAbs 3 and 10 reacted with the type 3- and type 3/14-specific MAbs, and the strain that reacted with PAbs 6 and 10 reacted with the type 6-specific MAbs. The two strains that reacted with PAbs 1 and 2 did not react with the type 1-specific MAbs; they probably do not belong to serotype 1.

To test the MAbs on cross-reactions with wild-type *U. urealyticum* strains, another 25 clinical isolates were serotyped with PAbs and MAbs. These strains were serotyped after only a few subcultures after primary isolation. On this limited number of strains, no cross-reactions between the MAbs and other *U. urealyticum* serotypes were observed. Unfortunately, not all 14 serotypes were represented among these 25 wild-type *U. urealyticum* strains.

None of the serotype 3 strains reacted only with MAb 3/14, indicating that serotype 14 strains were not misidentified as serotype 3 by PAbs. From these results, we can conclude that serotyping of clinical isolates with MAbs appears to be promising. Almost all PAb-monoreacting strains were accurately typed, whereas PAb-multireacting strains gave mixed results, indicating the better specificities of the MAbs that we developed. Since there are 14 serotypes of *U. urealyticum*, a further investigation of the serotype-specific antigens of *U. urealyticum* serotypes is needed and further evaluation with more serotype-specific MAbs on clinical strains should be performed.

To evaluate the antigenic variation in clinical isolates, 21 *U. urealyticum* clinical isolates (1 serotype 1, 14 serotype 3, and 6 serotype 6 strains) were further analyzed in immunoblots with the serotype 1-, 3-, and 6-specific MAbs. The strains were subcloned up to 10 times by the broth dilution method (10), and each subclone was tested by immunoblotting with the respective MAbs.

None of the serotype 1, 3, and 6 clinical isolates showed an immunoblot pattern similar to that observed with the reference strains, and almost all immunoblot patterns of the clinical isolates were different from each other, regardless of the number of passages performed before testing. The antigenic size variation in the clinical isolates had no influence on the reactivity by colony-IFA; all strains that reacted with the MAbs in the immunoblots also reacted with the same MAbs in the colony-IFA.

A comparison between the immunoblot patterns of serotype 3 clinical isolates performed before or after subcloning revealed that strains showing more than one band before subcloning were likely to have only a single band after subcloning (Fig. 1), suggesting the existence of a mixture of subpopulations before subcloning. Unlike serotype 3 clinical isolates, the immunoblot patterns of serotype 1 and 6 clinical isolates were rather similar before or after subcloning (data not shown).

Antigenic variation is quite common in mycoplasmas, both

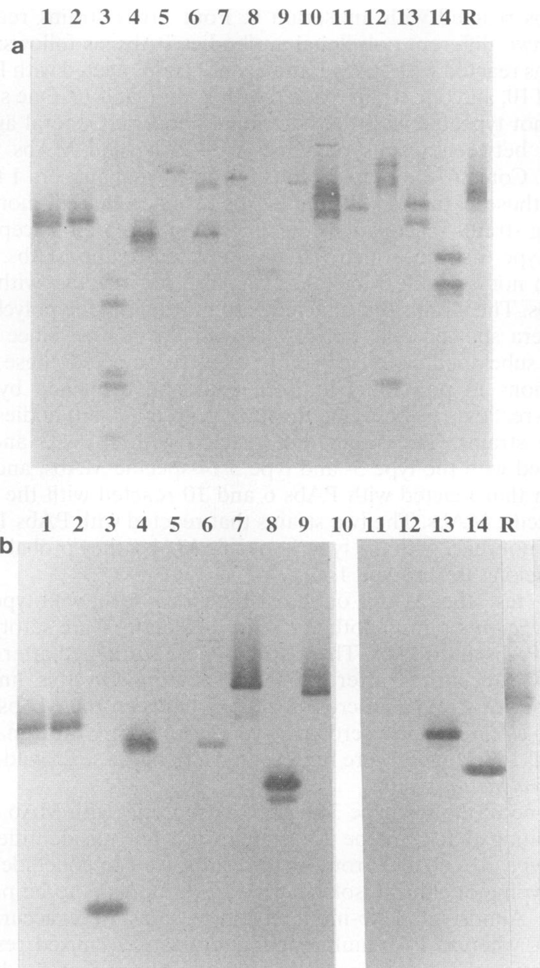

FIG. 1. Immunoblots showing reactions of *U. urealyticum* clinical isolates with serotype 3-specific MAb. (a) Strains before subcloning. (b) Strains after subcloning. The antigens in lanes 1 to 14 are clinical isolates B5, B53, Da21, DF18, DF24, E5, E20, G17, L12, L17, L29, P42, Ve30, and Ve58, respectively; lane R, serotype 3 reference strain. The approximate molecular masses of the antigens after subcloning (b) are as follow: B5, 57 kDa; B53, 58 kDa; Da21, 36 kDa; DF18, 55 kDa; E5, 80 and 56 kDa; E20, 72 kDa; G17, 49 and 46 kDa; L12, 70 kDa; P42, 75 kDa; Ve30, 56 kDa; Ve58, 51 kDa.

in vitro and in vivo (2, 7, 8, 15, 16). In *U. urealyticum* strains, antigenic variation was already reported in serotype 3 strains, but the numbers of strains studied was very small (2, 14, 16). We analyzed 21 strains of serotype 1, 3, and 6 clinical isolates of *U. urealyticum* in immunoblots before and after subcloning. Our results suggest that serotype 1-, 3-, and 6-specific antigens undergo a high degree of size variation. This antigenic variation is not limited to *U. urealyticum* serotypes 1, 3, and 6; we have shown in a previous study (3) that the serotype 4-specific antigen is also subjected to antigenic variation. All of the serotype 4 clinical isolates had immunoblot patterns different from each other and different from that of the serotype 4 reference strain.

In summary, serotype 1-, 3-, and 6-specific MAbs were produced in the present study. They appeared to have much higher specificities than PAbs and appear to be promising for serotyping clinical isolates, despite the antigenic size variation of *U. urealyticum*. The role of antigenic size variation of *U. urealyticum* in clinical strains and their significance for possible pathogenicity should be further evaluated.

REFERENCES

1. **Cassell, G. H., K. B. Waites, D. Crouse, P. T. Rudd, K. C. Canupp, S. Stagno, and G. R. Cutter.** 1988. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very low birth weight infants. Lancet **ii:**240–244.
2. **Cassell, G. H., K. B. Waites, H. L. Watson, D. T. Crouse, and R. Harasawa.** 1993. *Ureaplasma urealyticum* intrauterine infection: role in prematurity and disease in newborns. Clin. Microbiol. Rev. **6:**69–87.
3. **Cheng, X., A. Naessens, and S. Lauwers.** 1993. Identification and characterization of serotype 4-specific antigens of *Ureaplasma urealyticum* by use of monoclonal antibodies. Infect. Immun. **61:**2253–2256.
4. **Naessens, A., W. Foulon, J. Breynaert, and S. Lauwers.** 1988. Serotypes of *Ureaplasma urealyticum* isolated from normal pregnant women and patients with pregnancy complications. J. Clin. Microbiol. **26:**319–322.
5. **Naessens, A., W. Foulon, H. Cammu, A. Goossens, and S. Lauwers.** 1987. Epidemiology and pathogenesis of *Ureaplasma urealyticum* in pregnancy and early preterm labor. Scand. J. Obstet. Gynecol. **66:**513–516.
6. **Naessens, A., and S. Lauwers.** 1987. Modified indirect immunofluorescence test for serotyping large numbers of *Ureaplasma urealyticum* clinical isolates. J. Clin. Microbiol. **25:**191–192.
7. **Olson, L. D., C. A. Renshaw, S. W. Shane, and M. F. Barile.** 1991. Successive synovial *Mycoplasma hominis* isolates exhibit apparent antigenic variation. Infect. Immun. **59:**3327–3329.
8. **Olson, L. D., S. W. Shane, A. A. Kappas, T. M. Cunningham, P. S. Probst, and M. F. Barile.** 1991. Monoclonal antibodies to surface antigens of a pathogenic *Mycoplasma hominis* strain. Infect. Immun. **59:**1683–1689.
9. **Robertson, J. A., L. H. Honore, and G. W. Stemke.** 1986. Serotypes of *Ureaplasma urealyticum* in spontaneous abortion. Pediatr. Infect. Dis. **5:**S270–S272.
10. **Robertson, J. A., and G. W. Stemke.** 1982. Expanded serotyping scheme for *Ureaplasma urealyticum* strains isolated from humans. J. Clin. Microbiol. **15:**873–878.
11. **Stemke, G. W., and J. A. Robertson.** 1985. Problems associated with serotyping strains of *Ureaplasma urealyticum*. Diagn. Microbiol. Infect. Dis. **3:**311–320.
12. **Taylor-Robinson, D.** 1983. The role of mycoplasmas in nongonococcal urethritis: a review. Yale Biol. Med. **56:**537–543.
13. **Thirkell, D., A. D. Myles, and W. C. Russell.** 1989. Serotype 8- and serocluster-specific surface-expressed antigens of *Ureaplasma urealyticum*. Infect. Immun. **57:**1697–1701.
14. **Watson, H. L., D. K. Blalock, and G. H. Cassell.** 1990. Variable antigens of *Ureaplasma urealyticum* containing both serovar-specific and serovar-cross-reactive epitopes. Infect. Immun. **58:**3679–3688.
15. **Watson, H. L., L. S. McDaniel, D. K. Blalock, M. T. Fallon, and G. H. Cassell.** 1988. Heterogeneity among strains and a high rate of variation with strains of a major surface antigen of *Mycoplasma pulmonis*. Infect. Immun. **56:**1358–1363.
16. **Zheng, X., H. L. Watson, K. B. Waites, and G. H. Cassell.** 1992. Serotype diversity and antigen variation among invasive isolates of *Ureaplasma urealyticum* from neonates. Infect. Immun. **60:**3472–3474.