

Detection of *Mycoplasma genitalium*-Reactive Cervicovaginal Antibodies among Infected Women[∇]

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***Mycoplasma genitalium*-reactive cervicovaginal IgA and IgG antibodies were detected in 51.9% and 70.4% of 27 infected women and 22.2% and 18.5% of 27 uninfected controls, respectively. The predominance of MgpB- and MgpC-reactive antibodies at the site of infection is consistent with their hypothesized role in selecting antigenic variants during persistent infection.**

Mycoplasma genitalium is a sexually transmitted pathogen associated with urethritis in men and various inflammatory conditions in women, including cervicitis (reviewed in references 10 and 20). Additionally, *M. genitalium* is associated with chronic infection, as highlighted by retrospective longitudinal studies of urethritis (7) and cervicitis (3). Such persistence may aggravate the risk of adverse reproductive outcomes, including tubal-factor infertility and preterm birth, conditions associated with this organism (2, 6, 18). Circulating antibodies have been detected in the serum of men with *M. genitalium*-associated urethritis (19) and women with tubal-factor infertility (2, 18); however, the local antibody response in the lower genital tract remains uncharacterized. To better understand the immunopathogenesis of *M. genitalium*, we investigated the cervicovaginal antibody response at the initial site of infection.

To detect *M. genitalium*-reactive antibodies in the lower genital tract by immunoblotting, we used cervical and vaginal samples. These specimens were originally collected in our cross-sectional study of *M. genitalium* infection in women attending a sexually transmitted disease clinic (5). Infection status was determined using an *M. genitalium*-specific transcription-mediated amplification (TMA) assay (Gen-Probe, San Diego, CA) (22). In the protocol described below, samples from 27 *M. genitalium*-positive women were tested for reactivity to whole-cell *M. genitalium* G37 (ATCC 33530) by immunoblotting, with the next consecutive negative study participant serving as a control. All protocols were approved by the University of Washington Institutional Review Board.

We prepared G37 lysates from bacteria cultured in H broth (11), harvested by centrifugation, washed in phosphate-buffered saline (PBS), and boiled for 5 min in Novex Tris-glycine SDS sample buffer (Invitrogen, Carlsbad, CA) with 0.2 M dithiothreitol (DTT). The lysate (100 μg protein) was separated by electrophoresis using a single-well 7.5% SDS-polyacrylamide gel, and then proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Invitrogen). Membranes were incubated overnight (4°C) in blocking/dilu-

ent buffer (5% nonfat milk in PBS-0.1% Tween 20), cut into 5-mm strips, and reacted for 1 h with a 1:50 dilution of the cervical or vaginal specimen (swabs rehydrated in 2-sucrose-phosphate-based transport medium [c2SP]) (22). Membranes were washed (PBS-0.1% Tween 20) and incubated for another hour with peroxidase-conjugated goat anti-human IgG (whole molecule; Sigma-Aldrich, St. Louis, MO), IgA (alpha-chain; Sigma-Aldrich), or IgM (mu-chain; Sigma-Aldrich) diluted 1:10,000 or peroxidase-conjugated mouse anti-human IgG1, IgG2, IgG3, or IgG4 (Invitrogen) diluted 1:500. After another wash, membranes were developed with the Amersham ECL kit (GE Healthcare, Buckinghamshire, United Kingdom) and exposed to Kodak BioMax XAR film (Carestream Health, Rochester, NY); the appearance of bands after 20 min of exposure was interpreted as a positive result. To identify MgpB and MgpC, sera from rabbits immunized with His-tagged recombinant peptides made from fragments of either protein (S. L. Iverson-Cabral and P. A. Totten, unpublished data) were used with peroxidase-conjugated goat anti-rabbit IgG (whole molecule; Sigma-Aldrich), each diluted 1:10,000. Additionally, samples were reacted against lysates of *mgpB* and *mgpC* deletion mutants (1).

TABLE 1. Cervicovaginal immune response to *M. genitalium* among cases and controls

Immunoglobulin isotype	Sample site	No. (%) with reactivity (n = 27)		P value ^c
		Cases ^a	Controls ^b	
IgG	Cervix	19 (70.4)	4 (14.8)	<0.001
	Vagina	12 (44.4)	3 (11.1)	0.014
	Either site ^d	19 (70.4)	5 (18.5)	<0.001
IgA	Cervix	14 (51.9)	6 (22.2)	0.047
	Vagina	9 (33.3)	0 (0.0)	0.002
	Either site ^e	14 (51.9)	6 (22.2)	0.047

^a Women with *M. genitalium* DNA detected in both urine and vagina by TMA.

^b Women who were TMA-negative for *M. genitalium* DNA in the urine and vagina.

^c P values calculated by Fisher's exact two-tailed test.

^d Among cases, 63% were IgG positive in the cervix and vagina and 37% in the cervix alone. Among controls, 50% were IgG positive in the cervix and vagina, 25% in the cervix alone, and 25% in the vagina alone.

^e Among cases, 64% were IgA positive in the cervix and vagina and 36% in the cervix alone. All of the controls were IgA positive in the cervix only.

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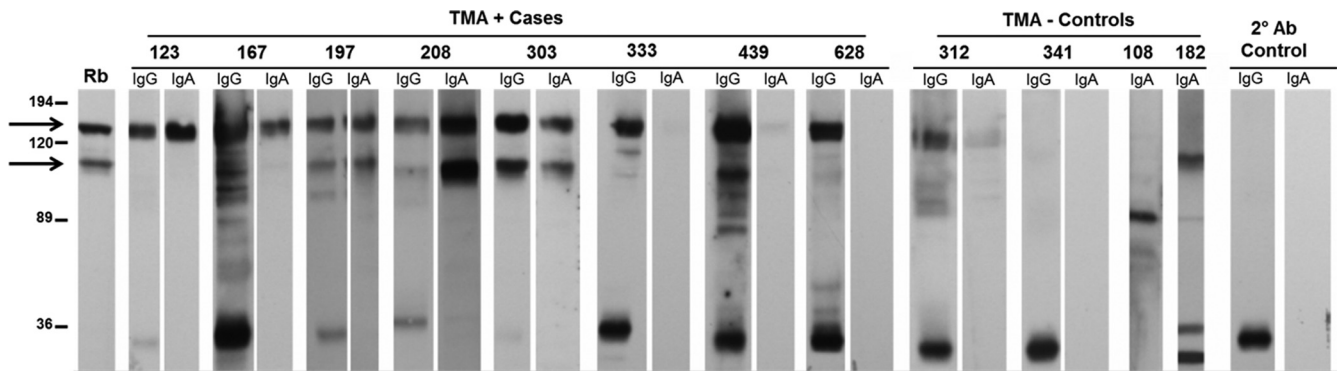


FIG. 1. Immunoblot analysis of *M. genitalium* cervical antibodies. Cervical results from representative cases and controls (study participant identification numbers listed above) demonstrating the range of reactivity observed against whole-cell *M. genitalium* G37 lysates. The locations of the immunodominant MgpB (140-kDa) and MgpC (110-kDa) proteins (arrows) were identified using a combination of sera from rabbits (designated “Rb”) immunized with His-tagged recombinant proteins spanning regions of MgpB (amino acids [aa] 1092 to 1209; GenBank accession number AAA25420) or MgpC (aa 36 to 308; GenBank accession number AAA25421). Membranes were exposed for 20 min; however, strongly IgG (participants 123, 197, 208, 303, and 629)- and IgA (participants 123 and 303)-reactive samples were exposed for 2 min to visualize individual bands. Secondary antibody controls in the absence of primary antibody indicated that an unknown *M. genitalium* protein (~36 kDa) reacts nonspecifically with anti-human IgG.

Infected women exhibited a cervicovaginal anti-*M. genitalium* response. *M. genitalium*-positive women were more likely to exhibit *M. genitalium*-reactive IgG and IgA than uninfected controls ($P = <0.001$ and 0.047 , respectively) (Table 1). While we were unable to identify *M. genitalium*-reactive IgM in cases or controls (data not shown), the IgG and IgA responses were pronounced (Fig. 1). The concordance for these isotypes among cases was relatively high, with both IgG and IgA detected in 74% and 50% of immunoblot-positive cervical and vaginal samples, respectively. To characterize the IgG response further, we isotyped a subset of the strongly IgG-positive specimens for which adequate sample volume was available: 11 (57.9%) and 7 (36.8%) of the 19 cervical and 3 (37.5%) and 1 (12.5%) of the eight vaginal samples tested were positive for IgG1 and IgG3, respectively (Fig. 2). Beyond the nonspecific binding of the IgG2 secondary antibody (Fig. 2), there was no evidence of an IgG2 or IgG4 response.

Although various *M. genitalium* proteins were antigenic (Fig. 1), antibodies against the 140-kDa MgpB and 110-kDa MgpC proteins were predominantly detected. These observations are consistent with previous immunologic studies of circulating antibodies in *M. genitalium*-infected individuals (2, 18, 19). Immunoblotting against lysates of *mgpB* and *mgpC* deletion mutants confirmed that these antigens were indeed MgpB and MgpC (Fig. 3). Among all immunoblot-positive women (including cases and controls), 92.3% (24/26) and 69.2% (18/26) had antibodies that recognized MgpB and MgpC, respectively; in fact, only two cervical samples from control women failed to react with MgpB and instead contained antibodies that targeted other, unknown *M. genitalium* antigens (participants 108 and 182) (Fig. 1). More detailed data regarding the antigenicity of MgpB and MgpC by sample site and antibody isotype are shown in Fig. 4.

To our knowledge, this is the first documentation of the

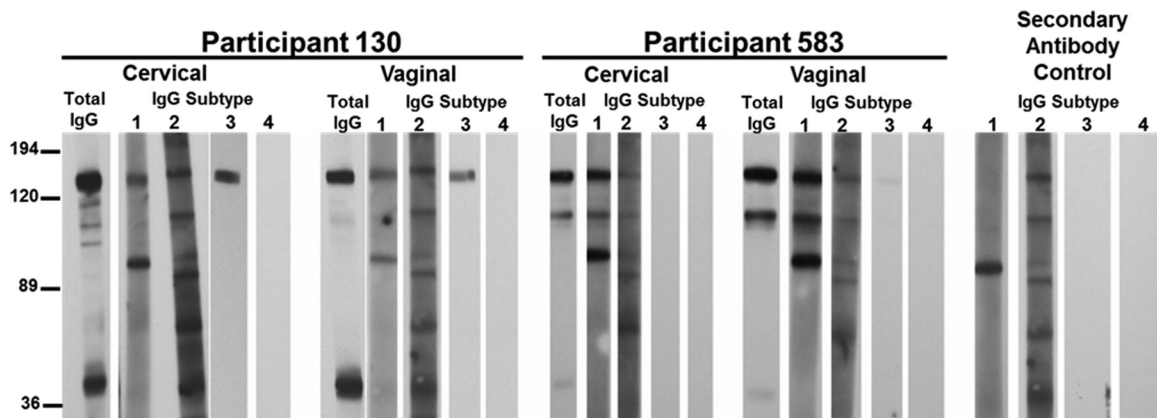


FIG. 2. *M. genitalium* infection elicits IgG1 and IgG3 subtypes. A subset of strongly IgG-positive samples was characterized further using subtype-specific secondary antibodies. The results from two IgG-positive cases are shown. Cervical and vaginal samples for participant 130 have IgG1 and IgG3 antibodies against MgpB, while participant 583 has a cervical and vaginal IgG1 response against MgpB and MgpC. Results using secondary antibody in the absence of primary antibody are included as a control showing the nonspecific cross-reactivity of anti-human IgG1 and IgG2.

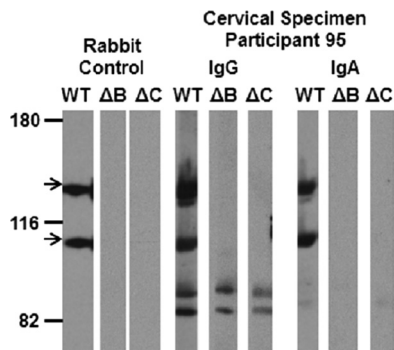


FIG. 3. Confirmation that the dominant *M. genitalium* antigens are the MgpB and MgpC proteins. The reactivities of MgpB and MgpC (arrows) were confirmed among a subset of immunoblot-positive women by reacting specimens with lysates of wild-type G37 (WT) or *mgpB* (ΔB) and *mgpC* (ΔC) deletion mutants. Immunoblot-positive samples failed to react with either antigen in the mutant lysates, because the MgpB and MgpC proteins are reciprocally stabilized; the *mgpB* mutant has reduced levels of MgpC, and the *mgpC* mutant has reduced levels of MgpB (1). Representative results are shown above, with the reactivity of participant 95 compared with that of the rabbit anti-MgpB and anti-MgpC control.

antibody response to *M. genitalium* in the lower genital tract. Our observations indicate that *M. genitalium* infection elicits a cervicovaginal IgA and IgG response, including IgG1 and IgG3 subtypes, with the MgpB and MgpC proteins immunogenic. Furthermore, the association between *M. genitalium* infection and the detection of IgG and IgA was significant. Differences in antibody response among infected women may reflect the site or duration of infection, previous exposure to this pathogen, or hormonal influences (12). The reactivity of some control specimens is likely due to prior *M. genitalium* infection or to nonspecific or cross-reactive antibodies, as likely observed with participants 108 and 182 (Fig. 2).

The biologic activity of *M. genitalium*-specific antibodies remains unknown. The isotypes identified in this study suggest that cervicovaginal antibodies may be involved in attachment-inhibition, complement-mediated killing, and/or opsonophagocytosis. IgG and IgA play an important role in mucosal defense and have different effector functions that work in concert to protect the host from infection. IgA serves as a first line of defense by preventing binding (17), interfering with motility, and enhancing opsonization through agglutination (21). The dominant isotype in cervicovaginal secretions is IgG (12, 14). Of the four isotypes, IgG1 and IgG3 are the most biologically active and proinflammatory through complement activation (15). These subtypes help control bacterial infections with opsonization and enhanced phagocytosis via the Fc receptor (4). In contrast, IgG4 lacks such effector functions (16), and IgG2 has low levels of complement activation (17), which is interesting given our negative results with these subtypes. We also failed to detect an IgM response, which may be explained by the low levels of IgM in genital secretions (14). Alternatively, these observations could be influenced by the timing of sample collection in the course of infection, as IgM is a short-lived isotype typically produced early in infection (17). Testing specimens collected closer to the onset of infection or selectively removing potentially interfering IgG from the sample may

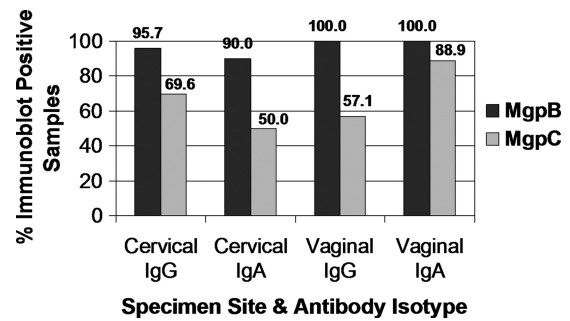


FIG. 4. Reactivity of immunoblot-positive specimens to the MgpB and MgpC proteins. The majority of immunoblot-positive samples contain antibodies that recognized MgpB and MgpC. All immunoblot-positive samples that demonstrated reactivity to *M. genitalium* G37 among cases and controls were used in this analysis, including 23 cervical and 14 vaginal IgG-positive samples and 20 cervical and 9 vaginal IgA-positive specimens. The percentage of samples with antibodies to MgpB (black) and MgpC (gray) is presented above. Among all immunoblot-positive specimens, only two fail to recognize MgpB (participants 108 and 182) (Fig. 1).

allow the detection of cervicovaginal IgM antibodies in future experiments.

Despite the induction of an antibody response at the initial site of infection, *M. genitalium* persists (3, 7), suggesting that this organism may have a mechanism(s) of immune evasion. It is noteworthy that the dominant antigens, MgpB and MgpC, are encoded by genes that exhibit extensive sequence diversity. Portions of *mgpB* and *mgpC* vary over time within *M. genitalium* G37 cultured *in vitro* and in samples collected longitudinally from individuals chronically infected with a single strain (8, 9, 13). We hypothesize that the evolution of sequence heterogeneity corresponds to changes in MgpB and MgpC antigenicity, allowing bacteria to escape the biologic activity of antibodies elicited by infection. Future studies are needed to characterize the biologic role of *M. genitalium*-specific antibodies and to correlate the development of antigenic diversity with the induction of these antibodies.

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