Humoral Circulatory Immune Response to *Gardnerella vaginalis*

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Received 23 January 1989/Accepted 7 June 1989

Strain-specific circulating immunoglobulin G and/or M was detected by enzyme-linked immunosorbent assay and immunofluorescence test by using Formol-treated suspensions of *Gardnerella vaginalis* from 28 women with overt vaginitis but only three symptom-free subjects among 43 otherwise healthy women found to be colonized by *G. vaginalis*. Analogous but less stringent strain specificity patterns were elicited by immunization of BALB/c mice.

*Gardnerella vaginalis*, a gram-variable, catalase- and oxidase-negative coccobacillus formerly known as *Haemophilus vaginalis* (7) or *Corynebacterium vaginale* (28), is a component of microbial consortia (19, 23–25) that can colonize the urogenital tracts of symptomless individuals but in many cases is associated with nonspecific vaginitis (vaginosis), i.e., abnormal vaginal discharge not due to yeasts or trichomonads (2, 10, 11, 14).

*G. vaginalis* has also been found in extravaginal foci of bacteremia, even in males (1, 4, 13, 15–19, 25), mostly in association with permissive conditions of the patients involved.

Vaginal infection sustained by noninvasive or conditionally invasive agents, such as *Trichomonas vaginalis* (21) and *Candida albicans* (20), respectively, can be associated with a systemic immune reaction. These considerations prompted the study of serum antibody reactions to *G. vaginalis* in vaginal infection and their correlation with the clinical picture. No data on this aspect of gardnerellosis have previously been published.

The study was extended to immunized mice as well. During a 19-month period, 505 fluid specimens were obtained at speculum examination from 280 nonpregnant women 18 to 41 years old attending the gynecological department of the hospital. Infection with *T. vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or herpes simplex virus and genital warts were ruled out by microscopic, cultural, and colposcopic examinations.

Medical histories and clinical data were recorded, the pHs of vaginal secretions were measured, and the fluids were microscopically examined for coccobacilli, granulocytes, and clue cells. The appearance of a fishy smell after treatment with 10% potassium hydroxide was noted.

All of the samples were immediately forwarded for isolation of the microorganism on selective human blood agar at 37°C in a 10% CO2 environment and subculture in brain heart infusion broth (Difco Laboratories). Identification was done as described by Yong and Thompson (27). Blood samples were obtained from *G. vaginalis*-infected subjects by cubital vein puncture, and sera were stored at −20°C. Surplus blood samples taken for unrelated diagnostic purposes from children and age-matched women found to be free of *G. vaginalis* were similarly processed and preserved as controls.

Groups of four BALB/c female mice (body weight, 18 g; Charles River Italia) were immunized by intramuscular and intraperitoneal injections of 0.5 ml of a repeatedly washed, Formol-treated bacterial suspension (5·10^9/ml from brain heart infusion broth culture) once a week for 4 weeks. No adjuvant was added. Blood was drawn from the retrobulbar plexus before and 1 week after the end of the immunization period. The sera of each group were pooled and stored at −20°C, as were control sera from sham-injected mice.

The enzyme-linked immunosorbent assay (ELISA) was done by standard procedures in 96-well plastic plates (1277 M129; Dynatech Laboratories, Inc.). The immunizing preparations of *G. vaginalis* and similarly treated suspensions of bacterial strains from the institute collection (*Haemophilus influenzae* M11, *H. parainfluenzae* M150, *Streptococcus pyogenes* M18, and *Corynebacterium xerosis* M40) were suspended with sodium bicarbonate buffer and used to coat the plastic wells.

Antibody preparations against either human or murine immunoglobulin M (IgM) or IgG (Virion) conjugated with horseradish peroxidase and a Pasteur LP 200 microprocessor reader at 620 nm were used. For immunofluorescence, test drops of an unfixed bacterial suspension were dried on a slide, treated with serially diluted human or murine serum, washed, treated with fluorescein-conjugated antibody against either human or murine IgM or IgG (Virion), and observed with a Leitz Dialux 20 EB microscope.

*G. vaginalis* was isolated from 43 subjects, including 28 patients with clinical signs of vaginitis and 15 symptomless women. None of the subjects showed clinical signs of extravaginal localization of *G. vaginalis*. Sera of patients with overt clinical symptoms contained IgM and/or IgG and reacted at a significant level (1/64 to 1/1,024) in the ELISA with the homologous bacterial strain (i.e., that isolated from the patient's secretion), but not with *G. vaginalis* strains isolated from other subjects or with other bacteria of the panel. IgG reactivity was present at a low titer (1/32) in only three symptomless women. The control sera were all negative.

In the present series of patients, no correlation was observed between the type or titer of circulating antibodies and the duration of illness (as inferred from medical history) or the amount of bacteria in the vaginal fluid (as estimated by microscopy).

The preimmunization and control mouse sera were negative, and no IgM antibody responses were detected by ELISA in immunized animals. In mice, strain-specific IgG responses predominated, with only a few incomplete cross-reaction patterns that did not allow us to outline a serotyping scheme.

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Immunofluorescence tests of infected subjects and immunized mice confirmed the strain specificity of antibody reactivity. The immunofluorescence reaction was sustained by IgM in humans and IgG in mice. This finding confirmed that for vaginal gardnerellosis, the rule valid for other communicable diseases holds true: infection can occur in the absence of overt disease (2, 24). This finding is not surprising, because symptomless carriage of other vaginitis agents, such as T. vaginalis and C. albicans, is a known phenomenon (18, 20). The present data showed, moreover, that vaginal colonization by G. vaginalis can elicit systemic immune reactions without clinically detectable extravaginal localization.

This appears to be the first aimed demonstration of this phenomenon, although findings that can be interpreted similarly are described elsewhere (see, e.g., reference 8).

A point of interest in the present study is the close correlation between circulating antibodies and overt signs of nonspecific vaginitis. Statistical analysis indicated that the probability of fortuitous coincidence was fairly low (<5%). Therefore, a pattern emerges whereby the two phenomena, namely, systemic immune reaction and appearance of clinical symptoms, can be considered to be connected. An analogy can be drawn, for what it is worth, with male trichomoniasis, in which circulating antibodies against T. vaginalis have been observed only in subjects with overt urethritis and never in symptom-free carriers (21).

The reactivity of circulating IgM and IgG antibodies to the homologous strain, i.e., the one isolated from each woman's own vaginal discharge, was selectively restricted in each woman. The mouse immunization study confirmed that most of the strains did not cross-react in either the ELISA or the immunofluorescence test, whereas in the ELISA incomplete cross-reactivity by a few strains was observed.

These data are consonant with the findings of precipitin tests performed with rabbit antisera (6, 12) but are apparently at variance with the data of others (9, 22, 26) who worked with polyvalent antiserum against G. vaginalis. Differences in antigen preparation and test methods could explain the differences, but the available data do all point to the conclusion that the main cause is the potential of G. vaginalis to present different immunodominating antigens. This property can hamper the development of a G. vaginalis serotyping scheme (12) and delay the solution of still controversial questions related not only to epidemiology but also to the natural history of G. vaginalis infection, such as the ambiguous role of the antibody reaction.

Antibodies indeed do not sustain a serum killing effect (3), are of doubtful activity in mediating opsonization, phagocytosis, and killing of G. vaginalis by human granulocytes (5), and now appear to be connected with the pathogenesis of symptomatic vaginitis.

LITERATURE CITED