Detection of a Species-specific Antigen of *Gardnerella vaginalis* by Western Blot Analysis

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Western blot analysis was used to identify antigenic components of *Gardnerella vaginalis*. Polypeptides bound to nitrocellulose membranes were probed with murine antisera raised to two strains of *G. vaginalis*, and antibody–antigen complexes were detected with ¹²⁵I-labelled anti-mouse immunoglobulin followed by autoradiography. Although there was inter-strain variation in immunogenic polypeptide profiles, all 23 strains of *G. vaginalis* examined contained a common antigen of molecular mass 41 kDa. This antigen was not found in any of six other bacterial genera.

INTRODUCTION

In 1955 Gardner and Dukes described a micro-organism which they believed was the aetiological agent of non-specific vaginitis (NSV). Subsequently, however, controversy has surrounded both its role in disease and its taxonomic status. When first described, it was assigned to the genus *Haemophilus*. Further investigation showed that the micro-organism could be grown without X and V factors, and Reyn *et al.* (1966) concluded that its electron microscopic appearance resembled that of a Gram-positive bacterium. On these grounds, it was reclassified as *Corynebacterium vaginale*, but as numerous investigators regarded the bacterium as being neither classical Gram-positive nor negative, it was assigned eventually to a novel genus and species, designated *Gardnerella vaginalis* (Greenwood & Pickett, 1980).

The morphological, cultural, biochemical and serological characteristics of *G. vaginalis* have been studied in some detail (Taylor-Robinson, 1984). Its antigenic analysis, however, has been almost totally ignored, apart from the study by Smaron & Vice (1974). They used an immunodiffusion technique and showed that all isolates of *G. vaginalis* examined had a common antigenic determinant. In this report we present the results of an investigation into the antigenic composition of *G. vaginalis* using the techniques of SDS-PAGE and Western blotting.

METHODS

Bacteria. Twenty-three strains of *G. vaginalis*, identified according to the criteria of Taylor & Phillips (1983) were studied. Strains 3:1, 16:1, 26:3, 33:4, 39:2, 40:1, 42:5, 47:3, 49:6, 54:1, 67:1, 76:1, 76:3 and 81:1 were obtained from E. Taylor, St Thomas' Hospital Medical School, London, UK, strains 661, 662, 663, 673, 6376, 8821 and 10915 from C. Ison, St Mary's Hospital Medical School, London, and strains 762 and 763 from the Praed Street Clinic, London. The bacteria were grown routinely at 37 °C for 48 h on Columbia agar containing 10% (v/v) defibrinated sheep blood (Difco) in an atmosphere of 5% (v/v) CO₂ in air. Then the organisms were removed from the agar with a bacteriological loop, suspended in sterile distilled water and washed by centrifugation. The pellet was resuspended in distilled water and the suspension stored at –20 °C. In several experiments, the bacteria were grown in peptone/starch/dextrose (PSD) broth (Dunkelberg *et al.*, 1970) containing 10% (v/v) heat-inactivated (56 °C for 30 min) horse serum. The organisms were washed twice by centrifugation, resuspended in a small volume of distilled water and stored at –20 °C. Bacteria ascribed to five other genera were also studied. These were: unclassified coryneforms 25:6 and 56:3 (from E. Taylor); *Neisseria gonorrhoeae* 203H (authors' isolate); *Haemophilus influenzae* strains 2104 (authors' isolate), Eagan (from T. Inzana, Washington State University, USA)

and H126 (from R. Wall, CRC, Harrow); *Campylobacter fetus* 3329 and *C. jejuni* 42739 (from A. O'Sullivan, CRC, Harrow); and *Bacteroides ureolyticus* W2N12 (from E. Fontaine, CRC, Harrow). Apart from *Neisseria gonorrhoeae*, which was grown on chocolate agar, and *Bacteroides ureolyticus*, which was grown under anaerobic conditions, all the other micro-organisms were grown on Columbia agar, which was incubated and the organisms harvested as described previously.

Preparation of cell extracts. Before use, 100 µl samples of each bacterial suspension were boiled for 3 min with 10 µl 25% (w/v) SDS and the protein concentrations of the mixtures were assayed by the Lowry method. Then the bacterial suspension and sample buffer (Laemmli, 1970) were added in equal volumes (maximum total volume of 100 µl) to give a protein concentration of 60 µg per sample. The preparations were boiled for 3–5 min, cooled, and particulate matter removed by centrifugation before applying to the gels.

SDS-PAGE. Whole-cell extracts were analysed on 10% (w/v) acrylamide slab gels, according to the method of Laemmli (1970). Protein standards ranging from 14.4 to 195 kDa (Bio-Rad) were run on each gel for molecular mass determinations and consistency comparisons. Gels were placed in a Protean I dual electrophoresis system (Bio-Rad) at a constant current of 30 mA per gel until the tracking dye (bromophenol blue) was 2 cm from the end of the gel. The gels were blotted as described below, or stained overnight with 0.1% (w/v) Coomassie Brilliant Blue R-250 (Bio-Rad) in water/methanol/acetic acid (5:5:2, by vol.) and destained in water/methanol/acetic acid (5:3:2, by vol.).

Western blotting. The separated cellular components on the gels were transferred electrophoretically in a Trans-Blot cell (Bio-Rad) onto a 0.2 µm nitrocellulose membrane (Schleicher & Schüll) as described by Towbin *et al.* (1979). After transfer, the outside lanes of the nitrocellulose filter, corresponding to the lanes of the gel that contained molecular mass markers, were removed, stained with 0.1% (w/v) naphthol blue black (Sigma) for 2 min and destained in the solution described above. The remaining nitrocellulose membrane was washed in a buffer solution of 1% (w/v) BSA, 0.001% (v/v) Tween 20 in PBS (NaCl, 8 g l⁻¹; KCl, 0.2 g l⁻¹; Na₂HPO₄, 1.5 g l⁻¹; KH₂PO₄, 0.2 g l⁻¹; pH 7.4) for 1 h to block any remaining free sites on the membrane. The membrane was then immersed for 2 h in *G. vaginalis* antiserum diluted 1:500 in the BSA buffer solution described above. After washing for 30 min with three changes of buffer, the membrane was probed for 1 h with ¹²⁵I-labelled rabbit anti-mouse immunoglobulins (Dako, Copenhagen), radiolabelled by the chloramine-T method (Greenwood *et al.*, 1963); specific radioactivity was 5 µCi (µg protein)⁻¹ [185 kBq (µg protein)⁻¹] and the antibodies were used at 2 × 10⁷ c.p.m. per blot. The membrane was then washed for 45 min in three changes of PBS, air-dried and exposed to Kodak X-Omat film with an intensifying screen at -70 °C for 1 d before developing.

***G. vaginalis* antisera.** Antisera to strains 47:3 and 673 were prepared in groups of 10 specific pathogen-free TO mice. Each mouse was inoculated intravenously with 0.2 ml of a freshly prepared suspension of *G. vaginalis* in PBS containing 10⁸ c.f.u. ml⁻¹, the organisms having been grown for 48 h at 37 °C as described previously. The inoculations were repeated 7 d later with another freshly prepared suspension, 3 d after which the mice were exsanguinated. The sera for each bacterial strain were pooled, separated by centrifugation and stored as small samples at -70 °C.

Serum antibodies to *G. vaginalis* were measured by a micro-immunofluorescence technique (Wang *et al.*, 1977), modified so that the organisms, suspended in PBS at a concentration of 1 × 10⁸ c.f.u. ml⁻¹, were not formalinized. The antibody titre was recorded as the reciprocal of the highest dilution of serum at which the bacteria fluoresced.

RESULTS

Western blotting

Twenty-three strains of *G. vaginalis* were examined in duplicate by Western blotting. Using ¹²⁵I-labelled anti-mouse immunoglobulins as the probe, immune mouse serum to strain 47:3, which had an immunofluorescent antibody titre of 512, recognized a wide variety of *G. vaginalis* proteins; the results of testing five representative strains are shown in Fig. 1. However, despite the variety of bands, all the strains of *G. vaginalis* had an immunodominant molecule which migrated to a distance corresponding to a molecular mass of 41 kDa (SD 0.76 kDa) (arrowed in Fig. 1). Furthermore, this antigenic molecule could be seen with extracts not only from organisms grown on agar medium, but also from those grown in broth. Antibodies to the various antigenic components, including the 41 kDa determinant, were not present in mouse serum as no immunogenic components could be found when blotting was done with serum from unimmunised mice.

Bacteria of other genera (see Methods), were examined to assess the specificity of the reaction with *G. vaginalis*. These bacteria were subjected to the same electrophoretic procedure and blotted with strain 47:3 *G. vaginalis* antiserum. Although some evidence of cross-reactivity was

Fig. 1. Western blot of *G. vaginalis* antisera 47:3 against five *G. vaginalis* strains.

Fig. 2. Western blot of *G. vaginalis* antisera 673 against *G. vaginalis* (Gv) and other organisms (Ng, *Neisseria gonorrhoeae*; Hi, *Haemophilus influenzae*).

Fig. 3. SDS-PAGE protein profiles of whole-cell homogenates of five strains of *G. vaginalis*.

seen between strain 47:3 of *G. vaginalis* and the other bacteria, none of them exhibited evidence of the 41 kDa determinant.

Mouse antiserum raised to a different strain of *G. vaginalis*, strain 673, was tested against the various strains of *G. vaginalis* and against the other bacteria. Several immunogenic molecules could be identified but, as shown in Fig. 2, *G. vaginalis* strains again exhibited an immunodominant molecule on the autoradiogram at 41 kDa (arrowed) which was not seen with the other bacteria. This molecule therefore appears to be a specific antigen shared by all strains of *G. vaginalis*.

Extracts of several *G. vaginalis* strains were incubated at 37 °C for 1 h with 50 µg Proteinase K (Boehringer) before application to the polyacrylamide gels. No immunogenic components could be seen when they were blotted with *G. vaginalis* antiserum, whereas extracts which had not been pre-treated, and on the same gel, exhibited characteristic profiles.

SDS-PAGE protein profiles

The value of Western blotting was highlighted by examining the protein profiles of the five *G. vaginalis* strains shown in Fig. 1 by SDS-PAGE before blotting. As shown in Fig. 3, the strains examined by this technique exhibited some heterogeneity but there was no common band migrating to a distance corresponding to 41 kDa which stained more heavily than any other band.

DISCUSSION

The Western blot technique has proved valuable in examining the antigenic composition of strains of *G. vaginalis*. Several proteins were revealed by probing with murine antiserum raised against the homologous strain, and other strains cross-reacted to varying extents. All strains

examined, however, shared a common antigen of 41 kDa. This cross-reactive molecule was present in *G. vaginalis* strains irrespective of their mode of growth and was not seen in any of the five other bacterial genera examined. Smaron & Vice (1974), using Ouchterlony analysis, showed that *G. vaginalis* strains possess a common antigenic determinant although their immunodiffusion technique gave no indication of its molecular mass.

The species-specific antigen defined by Western blotting was not a predominant band in Coomassie blue stained polyacrylamide gels. Since molecules other than proteins, such as lipopolysaccharides, are separated by SDS-PAGE, the question of the nature of the specific antigen arose. Some insight into its structure was obtained by protein digestion of the *G. vaginalis* extract prior to electrophoresis. Since bands did not develop in the lanes of the autoradiogram which contained digested material, it can be concluded that the antigen is partly, if not wholly, proteinaceous. Of course, our observations do not enable us to determine which of the antigens are exposed on the surface, since whole cells were homogenized. Furthermore the number of protein bands observed may be an underestimate of the antigenic complexity of the micro-organism because SDS usually denatures proteins, hence altering their antigenicity. Nevertheless, there is no doubt that a major antigen is common to all *G. vaginalis* strains. This opens the way for raising specific monoclonal antibodies which should not only aid in the diagnosis of *G. vaginalis* infections but also provide a tool for investigating whether the common antigen has a function in terms of attachment and hence pathogenicity.

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