

ANTIGENIC HETEROGENEITY OF GONOCOCCAL PILI\*

BY THOMAS M. BUCHANAN

(From the Immunology Research Laboratory, United States Public Health Service Hospital, and the Departments of Medicine and Pathobiology, The University of Washington, Seattle, Washington 98114, and The Rockefeller University, New York 10021)

Gonococcal pili were initially purified in 1972 (1). Studies with a fluorescein-conjugated globulin fraction of rabbit antiserum to purified gonococcal pili of strain 2686 suggested common antigenicity among gonococcal pili of 18 strains tested from the New York City area (2). Further evidence of shared antigens on many gonococcal pili was obtained with a radioactive antigen-binding assay using  $^{125}\text{I}$ -labeled purified gonococcal pili of strain 2686. This assay detected elevated antibody levels to 2686 pili in serum from 60 to 80% of patients with gonococcal infection (2, 3) and in serum from each of two groups of chimpanzees immunized with distinctly different strains of gonococci (4). However, no studies have been reported that directly compare purified pili from different strains of gonococci.

The development of a new purification procedure reported herein has allowed production of purified pili from two different gonococcal strains (2686 and 33), and direct comparison of their antigenicity in an antigen-binding assay. These studies indicate that pili of strains 2686 and 33 are distinguishable antigenically.

Materials and Methods

*Strains of Neisseria gonorrhoeae.* Two strains of gonococci were used; 2686 from Dr. Douglas Kellogg, Center for Disease Control, Atlanta, Ga. (2) and 33 from Dr. Kenneth Johnston, The Rockefeller University, New York. These two strains were of different gonococcal serotypes as tested and described by Johnston and Gotschlich (5). Different serotypes of gonococci were picked to insure that the gonococcal strains studied were distinctly different, since previous studies indicated antigenic similarity of pili from many gonococci (2, 3).

*Purification of Gonococcal Pili.* Colony type 2 gonococci were grown in liquid culture as previously described (2, 6). At the height of log phase growth, the liquid culture was briefly sheared in an omnimixer (Dupont Instruments, Sorval Operations, Newtown, Conn.). Organisms and large debris were removed from the sheared culture by centrifugation at 30,000 *g* for 30 min. The supernate was centrifuged at 150,000 *g* for 12-16 h. The pellet was resuspended in 0.01 M Tris buffer, pH 7.5, 0.01 M sodium azide, and centrifuged at 12,000 *g*. The supernate was mixed well with an equal volume of 20% saturated ammonium sulphate and left at 4°C overnight. The ammonium sulphate suspension was centrifuged at 30,000 *g* for 10 min and the precipitate was washed once in 10% saturated ammonium sulphate. After recentrifugation, the washed precipitate was redissolved in distilled water. The ammonium sulphate precipitation, washing, and centrifugation steps were repeated once and the precipitate was dissolved in distilled water so as to concentrate the original liquid culture 250- to 500-fold.

*Criteria for Purity of Gonococcal Pili.* Direct observation by electron microscopy and sodium dodecyl sulfate (SDS) polyacrylamide disc gel electrophoresis were used to assess the purity of

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gonococcal pili preparations from strains 2686 and 33 as previously described (2). Gels containing unlabeled samples were fixed and the SDS bleached out using 5.0% TCA. They were then stained using 0.025% aqueous Coomassie Brilliant Blue (Sigma Chemical Co., St. Louis, Mo.) in 7% acetic acid containing 45% methanol for 5-8 h, and destained in 7% acetic acid containing 10% methanol. Slab gels were run according to the techniques of Maizel (7) to simultaneously compare pili from strains 33 and 2686. After initial experiments showed that a single band of protein staining with Coomassie Blue was obtained which corresponded to the single peak of radioactivity for the radioactive pili samples, pili preparations were routinely assessed with radioactive samples so as to conserve pili. Pili preparations were labeled with  $^{125}\text{I}$  by the chloramine-T procedure of Greenwood et al. (8).

*Radioimmunoassay for Gonococcal Pili.* The radioimmunoassay utilized for gonococcal pili was as described previously (2). Approximately 100 ng of  $^{125}\text{I}$  labeled purified gonococcal pili from strain 2686 or 33 was reacted with a quantity of antiserum that contained approximately 2 ng of IgG-precipitating rabbit antibody to pili of strain 2686 or 33, respectively. This amount of antibody produced approximately 65% binding of the radiolabeled pili. To this constant amount of radiolabeled pili and antiserum in a series of microfuge tubes, was added incremental amounts of purified pili from either the same or a different gonococcal strain. Only pili that were pure by electron microscopy (Fig. 1) and SDS polyacrylamide gel electrophoresis were used in these studies. The radioisotope  $^{22}\text{Na}$  was added as a volume marker and the final volumes were equalized with 1% bovine serum albumin in phosphate-buffered saline, pH 7.2. After mixing well, the suspensions of antigen, antibody, and inhibitor were allowed to equilibrate overnight at 4°C. After equilibration, all antibodies in the rabbit antiserum were precipitated with goat antirabbit immunoglobulin antiserum. The percent binding of  $^{125}\text{I}$ -labeled pili antigen was determined after centrifugation, removal of supernate, and assessment of radioactivity in the precipitate as previously reported (2, 9). The inhibition of binding of the radiolabeled pili was calculated using the formula:

$$\% I = 100 \left( \frac{B_w - B_i}{B_w} \right)$$



FIG. 1. Purified gonococcal pili from strain 2686 ( $\times 125,000$ ). Pili from strains 33 and 2686 were morphologically indistinguishable. White dots are a grid-stain artifact.

where  $I$ , inhibition;  $B_w$ , binding without inhibitor; and  $B_i$ , binding in the presence of inhibitor. The protein concentration of purified pili preparations was determined using the Folin-Ciocalteu method as described by Lowry et al., using bovine plasma albumin (Metrix, Armour Pharmaceutical Co., Chicago, Ill.) as a standard (10).

### Results

Purified gonococcal pili from strains 2686 and 33 were obtained in yields of 50–100  $\mu\text{g/liter}$  of liquid culture using the purification procedure based on precipitation of gonococcal pili in 10% saturated ammonium sulphate. The SDS polyacrylamide gel electrophoresis patterns obtained for purified pili preparations from strains 2686 and 33 used in these studies are shown in Figs. 2 and 3, respectively. When these two pili preparations were electrophoresed in the same slab SDS polyacrylamide gel their subunit molecular weights were identical. Fig. 4 compares the inhibiting ability of purified gonococcal pili of strains 2686 and 33 in a binding system utilizing  $^{125}\text{I}$ -labeled 2686 pili and antibody to 2686 pili. Nonlabeled pili from strain 2686 progressively inhibited the assay with increments of 320–3,200 ng of pili. In contrast, no significant inhibition of the assay was produced with equal amounts of purified pili from strain 33 (Fig. 4). Fig. 5 compares the inhibiting ability of purified gonococcal pili of strains 2686 and 33 for an immunoassay utilizing  $^{125}\text{I}$ -labeled pili from strain 33 and antibody to strain 33 pili. Progressive inhibition of binding of labeled pili from strain 33 was produced with 50- to 2,500-ng increments of nonlabeled purified pili from the same strain (Fig. 5). Purified pili from strain 2686 failed to produce significant

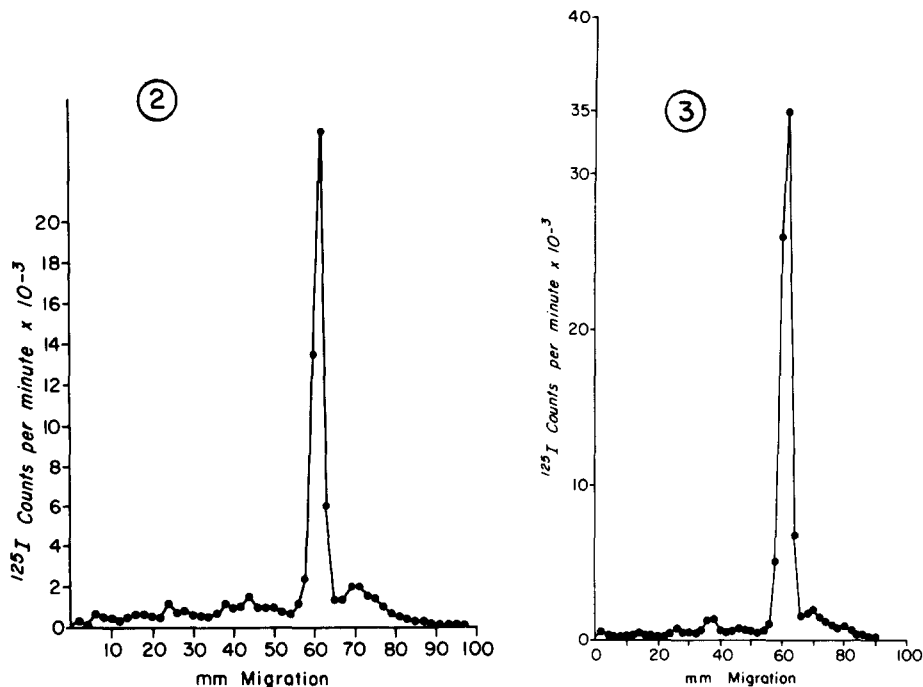


FIG. 2. SDS 10% polyacrylamide gel pattern of  $^{125}\text{I}$ -labeled purified pili from strain 2686.

FIG. 3. SDS 10% polyacrylamide gel pattern of  $^{125}\text{I}$ -labeled purified pili from strain 33.

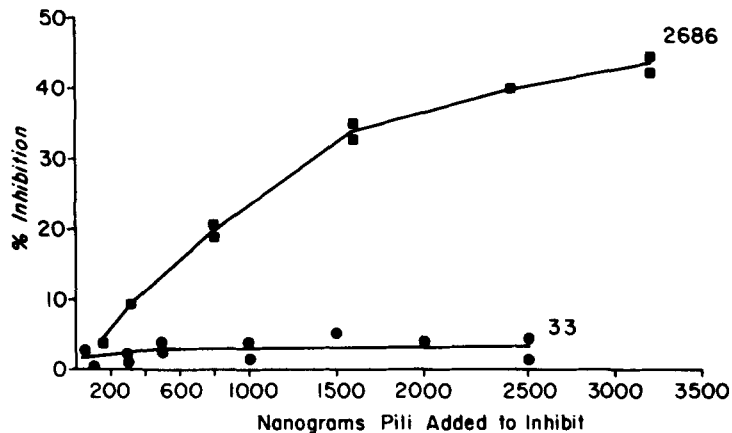


FIG. 4. Pili radioimmunoassay with  $^{125}\text{I}$ -labeled 2686 pili and antiserum to 2686 pili. Inhibition with purified pili from strains 2686 and 33.

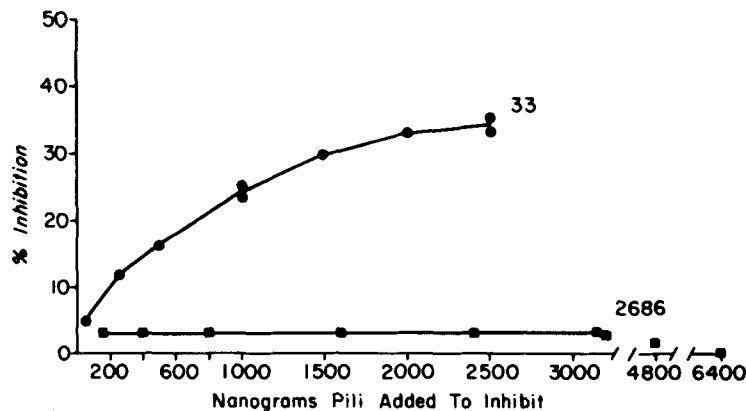


FIG. 5. Pili radioimmunoassay with  $^{125}\text{I}$ -labeled 33 pili and antiserum to 33 pili. Inhibition with purified pili from strains 33 and 2686.

inhibition of this assay even in amounts as great as 6,400 ng, a 64-fold excess over the amount of labeled strain 33 pili.

### Discussion

These results indicate that the pili of strains 2686 and 33 are antigenically different. Some common antigens may be present on the two pili types as indicated by nearly maximal binding of labeled pili preparations by high titered antiserum to pili of either strain 2686 or 33. However, most of the antigenic sites on pili strain 2686 appear different from those on strain 33 pili when compared on a weight basis as illustrated in Figs. 4 and 5. This type of radioimmunoassay analysis allows clear differentiation of the two pili types.

The recognition that gonococcal pili vary antigenically between some strains may facilitate studies to determine the genetic control of piliation in gonococci. For example, transformation of strain 33 to produce pili of strain 2686 by DNA from strain 2686 could be detected by radioimmunoassay evaluation of the pili

produced by the transformed gonococci. Further antigenic types of gonococcal pili are likely to exist and serologic typing of gonococci based on different antigenic types of gonococcal pili may prove useful as an epidemiologic marker. Since gonococcal pili are sufficiently pure to be used as a radiolabeled antigen in antigen-binding assays, it may also be possible to determine the specific antigenic type of pili produced by gonococci infecting a given patient by quantitating the patient's antibody response to each separate gonococcal pili type. Antigenic types of pili produced by gonococci may differ by geographic area and with regard to clinical syndromes of gonococcal infection. The sensitivity of tests to detect antibody to pili in patients with gonorrhoea may be increased by including more than one antigenic type of gonococcal pili within the antigen preparations.

### Summary

Pili were isolated from two different strains of gonococci (33 and 2686) and demonstrated to be pure by the criteria of electron microscopy and polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Each purified pili preparation was studied for its ability to inhibit; (a) binding of  $^{125}\text{I}$ -labeled purified 2686 pili by antibody to 2686 pili, and (b) binding of  $^{125}\text{I}$ -labeled purified 33 pili by antibody to 33 pili. In each instance progressive inhibition of binding was produced by the homologous pili type, but no significant inhibition was observed using comparable weights of the heterologous pili type. These results indicate that the pili of strains 2686 and 33 are antigenically different.

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