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Monoclonal Antibodies to Outer Membrane Protein PII Block Interactions of *Neisseria gonorrhoeae* with Human Neutrophils

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Nonopsonic binding of gonococci to human neutrophils appears to be mediated by a family of heat-modifiable outer membrane proteins termed protein IIs (PIIs). We studied the ability of a wide variety of antigonococcal monoclonal antibodies (MAbs) to inhibit the interactions of nonpiliated PII$^+$ gonococci with human neutrophils by measuring gonococcal adherence to neutrophils and subsequent luminol-enhanced neutrophil chemiluminescence. From one set of 95 MAbs reacting with whole gonococci, only two, 7VA2 and 7B9, inhibited the ability of gonococci to induce neutrophil chemiluminescence. 7VA2 and 7B9 both reacted only with PII. MAb 53C4, from a smaller set of anti-PII MAbs, inhibited adherence to neutrophils of PII variants that bound 53C4, but not of PII variants that did not. It also inhibited gonococcus-induced neutrophil chemiluminescence. Using a whole-cell binding assay and Western blotting (immunoblotting), we showed that MAb 53C4 bound to one PII (PII4) of strain F62 and to two PIIs (PIIb and PIId) of strain FA1090. The present studies confirm and extend the role of PII in gonococcal adherence to and stimulation of human neutrophils and show intrastrain conservation of PII epitopes. The results indicate that PII is the only outer membrane component involved in adherence of nonpiliated gonococci to human neutrophils.

The adherence of gonococci to neutrophils, the most abundant cells at local sites of disease, has been the subject of many studies and reviews (7, 10, 29, 35, 39, 40, 43). Three antigenically variable surface components, pili, lipopolysaccharide, and outer membrane protein II (PII), have been implicated as gonococcal adhesins for neutrophil receptors. PIIs undergo antigenic and phase variation at a frequency of 0.2×10^{-3} to 4.0×10^{-3} (20, 21, 30). This variation leads to the addition or deletion of one or more PIIs upon daily clonal subculture, which in turn leads to the formation of colonies of varying opacity. This constant switching of colonial morphology dictates cautious choice of gonococcal variants for study. The PII content of gonococci under study must be monitored routinely by several complementary methods.

The original leukocyte association protein described by Swanson and colleagues (14, 37) was identifiable as a PII protein, but was not associated with opacity. It is now recognized that gonococci expressing certain PIIs do not form obviously opaque colonies, but still adhere to neutrophils and activate the oxidative burst in the process of phagocytosis (25, 32, 42, 43). Conversely, Fischer and Rest described a gonococcal variant expressing a very opaque phenotype, FA1090 PIIa, that neither adheres to nor stimulates human neutrophils (9). Thus, gonococcal colony opacity cannot be directly related to neutrophil adherence.

Several studies with hyperimmune rabbit (34, 36) and convalescent human (17, 44) sera documented the immunogenicity and lack of cross-reactivity of native PIIs and suggested unique surface-exposed PII epitopes. No anti-PII monoclonal antibodies (MAbs) have been described that recognize all or most PIIs on whole gonococci (1, 23, 42). In contrast, structural data and nucleotide sequence information suggest that surface-exposed common peptides indeed exist among variant PIIs (6, 12, 30, 33). Thus, there may be common surface-exposed PII peptide sequences which, although available for iodination, are either nonimmunogenic or not physically accessible to bind antibody. Such regions might be involved in adherence of PII to neutrophils.

To confirm the role of PII in gonococcal adherence to human neutrophils, we studied the effects of several MAbs raised against gonococcal strain F62 on the nonopsonic interaction of gonococci with neutrophils. Our data indicate that PII-specific MAb epitopes are more conserved than previously thought, that anti-PII MAbs inhibit gonococcus-neutrophil interactions, and that PII is the only neutrophil adhesin on nonpiliated gonococci.

MATERIALS AND METHODS

Gonococci. Only nonpiliated gonococci were used in these studies. Gonococcal strains were maintained by daily clonal transfer on GC medium base agar (Difco Laboratories, Detroit, Mich.) with supplements (25). For use in adherence and chemiluminescence (CL) assays, bacteria were grown to log phase in gonococcal broth (25), washed once, suspended to 2×10^8 CFU/ml in Dulbecco phosphate-buffered saline (PBS) with 0.1% (wt/vol) gelatin (PBSG), and maintained at room temperature. PBS contained, per liter, 8.0 g of NaCl, 0.2 g of KCl, 1.15 g of Na_2HPO_4, 0.1 g of $CaCl_2$, and 0.1 g of $MgCl_2$, at pH 7.4. Colony types were identified by the criteria of Kellogg et al. (13) and Swanson (32). Strain F62 was obtained from P. Fred Sparling, University of North Carolina, Chapel Hill. FA1090 PII variants were obtained from Janne Cannon, University of North Carolina, Chapel Hill. Variants F62 PII2 and PII4 contained PIIs which imparted identical opacity phenotypes and did not clump in liquid culture or buffer. Variant F62 PII3 had an opaque friable colony and clumped profusely; consequently, no adherence data are shown for this variant. Variant FA1090 PIIb did not clump and had a transparent phenotype which could easily be confused with PII$^-$ variants. To verify that gonococci were of the correct PII type, whole-cell binding

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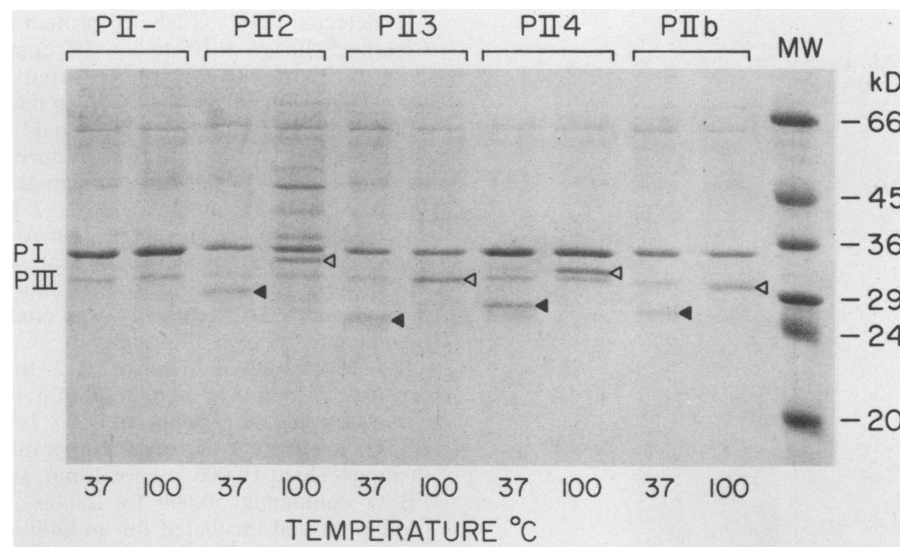

FIG. 1. Coomassie blue-stained SDS-PAGE of outer membrane preparations of PII variants used in this study. Lanes 1 through 8 contain outer membranes of F62 PII variants, and lanes 9 and 10 contain outer membranes from variant FA1090 PIIb. The left sample of each pair was solubilized at 37°C for 1 h, whereas the right sample was boiled for 5 min prior to electrophoresis. The closed and open triangles indicate the location of unheated and heated PIIs, respectively. kD, Kilodaltons.

assays, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of outer membranes, or Western blotting (immunoblotting) was performed on a routine basis as described below. Figure 1 shows the SDS-PAGE profiles of outer membranes from F62 PII variants used in this study. The mobility of FA1090 PIIs has been described before (1, 9). FA1090 PIIb is shown in Fig. 1 for comparison with the F62 PIIs.

Neutrophils. Neutrophils, purified through Ficoll-Hypaque by the method of Ferrante and Thong (8), were suspended to 10^7/ml in PBSG without calcium or magnesium and maintained on ice.

MAbs. Milton R. Tam generously supplied 95 MAbs as ascites fluids (Table 1). These MAbs were raised against several gonococcal strains including F62 and included MAbs to PIA, PIB, PII, PIII, lipopolysaccharides, and presumably other uncharacterized gonococcal antigens (15, 38). MAb 3G11 is from this collection (Fig. 2) and was specific for a reduction-modifiable molecule of 30 to 31 kilodaltons. The antigen recognized by 3G11 was present in strains F62 and FA1090 regardless of PII content and is presumably PIII. Two MAbs to PII variants of strain F62 were kindly supplied by Charles E. Wilde, University of Indiana, Indianapolis, as ascites fluids (23). Janne Cannon generously supplied several anti-PII hybridoma supernatants containing MAbs against strain FA1090 (1). These MAbs were used to ascertain correct PII variants of strains, but were not studied in CL or adherence assays. Samples of all MAbs were kept frozen at −70°C and, once thawed, were not refrozen.

CL. Luminol-enhanced neutrophil CL was measured as described previously (25, 41). Screening of MAbs for their ability to inhibit gonococcus-induced CL was done by preincubating gonococci with dilutions of ascites at room temperature for 15 min before addition of neutrophils.

TABLE 1. MAbs used in this study

Source	Immunizing gonococcal strain	Specificity	Designation	PII variants recognized by MAb	Reference(s)
M. Tam	F62 and others	Assorted gonococcal immunogens	7VA2	F62 PII3[a]	23
			7B9	F62 PII3[a]	24
C. Wilde	F62	PIIs not characterized	53C4 (−1)	F62 PII4[a]	18
				FA1090 PIIb[b]	
				FA1090 PIId[b]	
J. Cannon	FA1090	PIIs characterized	10.1 (H4)	FA1090 PIIb[b]	19, 24
				FA1090 PIId[b]	26, 40
				F62 PII4[a]	
			H138	FA1090 PIIa[b]	
			H157	FA1090 PIIc[b]	
			H164	FA1090 PIIe[b]	
				F62 PII2[a]	
			H156	FA1090 PIIf[b]	

[a] Our PII variant designations.
[b] PII variant designations by Cannon and colleagues (19, 22, 40).

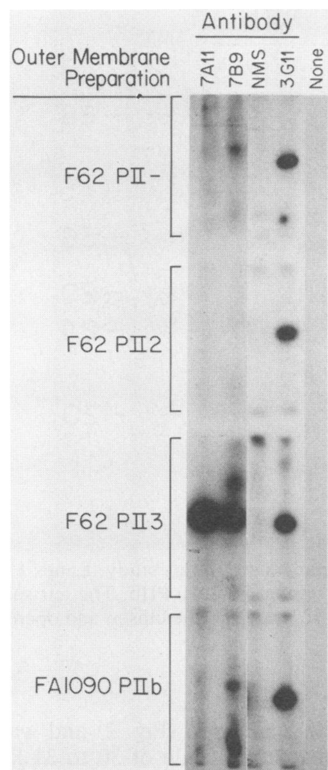

FIG. 2. Western blotting of outer membranes from gonococcal PII variants. Four gels (12%), each poured with a single preparative well comb, were loaded with approximately 20 μg of the indicated outer membrane preparations. After electrophoresis and electrotransfer, the nitrocellulose transfers were cut into strips and probed with appropriate dilutions of antibodies as indicated for each lane. After washing, antibody binding was detected with ^{125}I-labeled protein A and radioautography. NMS, Normal mouse serum. None indicates no primary antibody.

Adherence assay. Gonococci at 2×10^8 CFU/ml were incubated with dilutions of MAbs for 15 min at room temperature before their addition (125 μl) to 5×10^5 neutrophils (pretreated with 5 μg of cytochalasin B per ml for 10 min at 37°C to prevent phagocytosis) in a final volume of 1 ml of PBSG, resulting in a gonococcus/neutrophil ratio of 50:1 (9). Ratios of >50:1 induced extensive clumping of neutrophils. Gonococci and neutrophils were incubated in sterile snap-cap plastic tubes (10 by 50 mm) on a rotator at 10 rpm for 30 min at 37°C (24). At 30 min, 150 μl of the gonococcus-neutrophil mixtures was placed in a cytocentrifuge (Shandon Southern) and spun at a setting of 6 for 10 min. The resulting slides were stained with Wright stain, and 100 contiguous neutrophils were counted. Adherence data are expressed in two ways: (i) the percentage of neutrophils with 0, 1 to 2, 3 to 5, 6 to 8, or >8 adherent gonococci; and (ii) the average number of gonococci per 100 neutrophils.

Western blotting. To detect which antigens were recognized by MAbs, Western blotting of gonococcal outer membranes (prepared with $LiCl_2$ as described by Leith and Morse [19]) was performed as described by Bszewczyki and Koloft (4). Outer membranes were heated at 100°C for 5 min in Laemmli sample buffer and resolved on an SDS–12% polyacrylamide slab gel, using the Laemmli buffer system in a minigel apparatus (Bio-Rad Laboratories, Richmond, Calif.) before electrotransfer with a Genie rapid electroblotting apparatus (Idea Scientific, Corvallis, Ore.) (16). Bound antibody was detected with ^{125}I-labeled protein A (Amersham Corp., Clearfield, Ill.), 1×10^6 to 2×10^6 cpm per 9- by 5-cm blot. Prior to exposure to X-ray film, the nitrocellulose was stained for protein with amido black (26) so that a direct comparison of film bands with protein bands could be made.

Colony lifts. A colony lift procedure was performed on a mixture of F62 PII variants by the method of Black et al. (2). Primary antibody binding was for 2 h, and antibody was detected by using a 1:1,000 protein A-alkaline phosphatase conjugate (Sigma), using the substrate described by Blake et al. (3). Reactive colonies were subcultured by single-colony passage, and MAb reactivity was confirmed by whole-cell binding and Western blotting.

Whole-cell binding of MAbs. A 2- to 5-μl amount of precentrifuged ascites or 10 to 50 μl of hybridoma supernatants was added to 1 ml of gonococci (2×10^8 gonococci per ml in PBSG) for 15 min at room temperature. Gonococci were then pelleted at $10{,}000 \times g$ for 1 min, suspended in 1.0 ml of PBSG containing 50,000 to 100,000 cpm of ^{125}I-labeled protein A, and incubated for an additional 15 min at room temperature. The bacteria were then pelleted, washed once in PBSG, and counted in a gamma counter. Binding of MAbs to homologous PII^+ variants was usually 10-fold that of binding to PII^- variants and was often more than half the starting counts. Controls lacking either gonococci or primary antibody contained <1,000 cpm (data not shown). Similar assays have been described for other purposes (5, 31).

ELISA. Screening of M. R. Tam's MAbs for reactivity to strain F62 PII3 and FA1090 PIIb was done by enzyme-linked immunosorbent assay (ELISA) as described by Nachamkin et al. (22). MAbs exhibiting absorbance values of >0.05 were used with their homologous ELISA-reactive strains in CL assays.

Radioimmunoprecipitation. Presolubilized radioimmunoprecipitation was performed as described by Hansen et al. (11). Gonococcal outer membranes were iodinated, using Iodobeads (Pierce Chemical Co., Rockford, Ill.) and ^{125}I (product IMS-30; Amersham) as described by the manufacturer. After iodination, membranes were solubilized in 1% (wt/vol) Zwittergent 3.14 (Calbiochem-Behring, La Jolla, Calif.) in 20 mM Tris–50 mM NaCl–5 mM EDTA (TNE) for 30 min at 37°C with occasional vigorous vortexing with glass beads. Membranes were centrifuged at $100{,}000 \times g$ for 1 h at 4°C. The supernatant was chromatographed through Sephadex G-10 preequilibrated with 0.1% Zwittergent 3.14 in TNE or dialyzed overnight against 0.1% Zwittergent 3.14 in TNE to remove free iodine. When analyzed by SDS-PAGE, this supernatant contained all three major outer membrane proteins (PI, PII, and PIII) and several minor ones.

Fab preparation. Preparation of antibody-binding fragments (Fabs) was performed by using a kit from Pierce Chemical Co. and following the manufacturer's recommendations. Each step in the purification was monitored by nonreducing SDS-PAGE, using 8% (for immunoglobulin G) or 12% (for Fabs) acrylamide gels. Fabs were dialyzed against PBS prior to use. Activity was verified by ELISA, using whole gonococci as antigen and goat anti-mouse immunoglobulin conjugated to alkaline phosphatase (1:500) (Sigma) as the secondary antibody (22).

RESULTS

Initial screening of antigonococcal MAbs. We screened a set of 95 MAbs raised against whole gonococci (Table 1) to identify surface-exposed antigenic determinants important in gonococcus-neutrophil interactions and to confirm the im-

TABLE 2. Inhibition by MAbs 7VA2 and 7B9 of gonococcus-induced neutrophil CL[a]

MAb	MAb titer	Peak CL, cpm (10^3) (%)	
		F62 PII3	F62 PII2
None		27 (100)	545 (100)
7VA2	100	13 (47)	626 (115)
	1,000	14 (51)	
7B9	100	9 (34)	613 (112)
	1,000	16 (59)	

[a] Gonococci were incubated with MAbs at room temperature for 15 min and then used to stimulate neutrophil CL. Values in parentheses indicate percentage of controls containing no MAbs. Results for experiments with MAbs are the means of duplicates, and control values are the means of four determinations. Results shown are typical of data from two other experiments.

portance of PII in such interactions. ELISA screening of these MAbs with gonococcal variant F62 PII3 whole cells yielded 46 MAbs that reacted with an optical density of >0.05. Forty-two of these MAbs reacted with gonococcal variant FA1090 PIIb (data not shown). MAbs positive in ELISA were screened for their ability to inhibit gonococcus-induced neutrophil CL, using homologous gonococcal variants.

Inhibition by MAbs of gonococcus-induced neutrophil CL. With the knowledge that PII-containing gonococci induce luminol-enhanced neutrophil CL (25), we investigated the ability of the above 46 anti-F62 PII3 MAbs to inhibit such interactions. Only MAbs 7VA2 and 7B9 inhibited gonococcus-induced neutrophil CL. Inhibition was dose responsive (Table 2). MAb 7B9 consistently inhibited CL to a greater degree than MAb 7VA2. None of the 46 MAbs inhibited CL induced by gonococcal variant FA1090 PIIb.

Fabs were made from MAb 7B9 to study its specificity further and to reduce the possibility of steric effects or aggregation of organisms by whole immunoglobulin G. Variant F62 PII3, incubated for 15 min at room temperature with 5 μg of 7B9 Fab per ml, induced only 37% of the CL induced by untreated gonococci (mean of two experiments). Thus, Fab fragments of MAb 7B9 specifically inhibited CL to a similar degree as whole MAb 7B9.

Antigens recognized by 7VA2 and 7B9. We used Western blotting and presolubilized radioimmunoprecipitation to identify gonococcal antigens recognized by MAbs 7VA2 and 7B9. MAbs 7VA2 and 7B9 reacted strongly in Western blotting only with PII3 of strain F62 (Fig. 2). They failed to react with outer membrane proteins from F62 PII$^-$, F62 PII2, or FA1090 PIIb. In contrast, MAb 3G11 recognized the

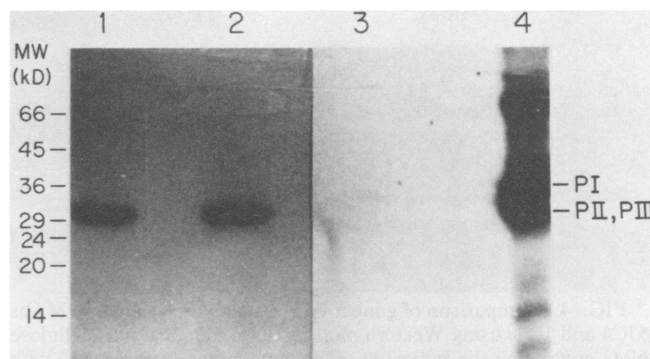

FIG. 3. Radioimmunoprecipitation of presolubilized gonococcal variant F62 PII3 outer membranes. Iodinated gonococcal membranes were immunoprecipitated with MAb 7VA2 or 7B9 and protein A-Sepharose. Following electrophoresis, the gel was dried and radioautographed. Lane 1, MAb 7VA2; lane 2, MAb 7B9; lane 3, no MAb; lane 4, Zwittergent soluble starting material. kD, Kilodaltons.

PIII of all four gonococcal PII variants (Fig. 2). MAb 3G11 appears to be PIII specific since it recognizes a reduction-modifiable antigen of 30 to 31 kilodaltons in Western blots (data not shown). It was used as a positive control to discriminate between PII and PIII, since F62 PII3 migrates very close to F62 PIII in SDS-PAGE (Fig. 1).

Radioimmunoprecipitation of presolubilized gonococcal outer membranes confirmed the results of Western blotting for MAbs 7VA2 and 7B9 (Fig. 3). Both MAbs strongly precipitated PII3 from strain F62.

Characterization of MAb 53C4. We also wished to see whether anti-PII MAb 53C4 (23), from a different set of MAbs than those discussed immediately above (Table 1), inhibited gonococcus-neutrophil interactions. Initial studies, however, revealed that none of the F62 PII variants used in our laboratory reacted in a whole-cell binding assay with 53C4. To find MAb-reactive PII clones, we grew multiple colony types on a single agar plate (with about 1,000 colonies) and screened them by colony blot with MAb 53C4. Three clones reacted with MAb 53C4. Subsequent studies (Western blotting, colonial morphology, and whole-cell binding) showed the three reactive clones to be identical. We designated the 53C4-reactive clone as variant F62 PII4.

Before using MAb 53C4 in biological assays, we characterized its binding to our various well-defined PII variants, using whole-cell binding assays. Results include data with MAb 53C4 as well as other anti-PII MAbs used in this study

TABLE 3. Binding of MAbs to whole gonococci[a]

Gonococcal variant	cpm bound (% of input cpm) to given MAb				
	10.1	53C4	7VA2	7B9	H164
F62 PII$^-$	ND[b]	350 (1)	190 (<1)	390 (1)	ND
F62 PII2	ND	470 (1)	160 (<1)	360 (1)	14,240 (28)
F62 PII3	ND	600 (1)	24,230 (48)	20,180 (40)	ND
F62 PII4	8,900 (18)	11,850 (24)	440 (1)	420 (1)	1,720 (3)
FA1090 PII$^-$	570 (1)	1,320 (3)	ND	170 (<1)	ND
FA1090 PIIb	11,930 (24)	21,490 (43)	ND	270 (1)	ND

[a] Gonococci were incubated with MAbs for 15 min and washed, and 50,000 cpm of ^{125}I-labeled protein A was added; incubation was for 15 min. Gonococci were then washed again and counted. See text for details. Data are from a single experiment.

[b] ND, Not determined in this experiment. Data from other experiments were negative for binding with this combination of MAb and PII variant.

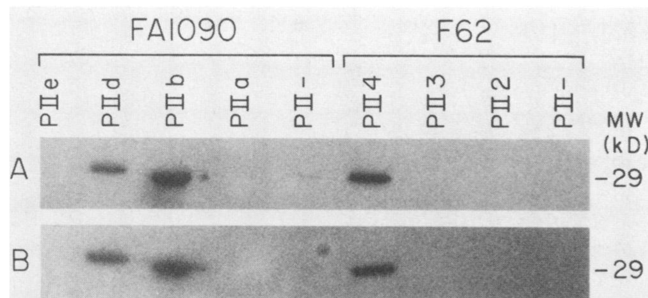

FIG. 4. Comparison of gonococcal variants recognized by MAbs 53C4 and 10.1, using Western blotting. Two identical nitrocellulose blots containing the indicated outer membranes were probed with MAb 53C4 (A) or MAb 10.1 (B). After washing, antibody binding was detected with ^{125}I-labeled protein A and radioautography. kD, Kilodaltons.

(Table 3). As expected, 53C4 bound to variant F62 PII4; it reacted with no F62 PII variants tested, suggesting a unique PII epitope described by others (1). We were surprised, however, to find that 53C4 bound to the PIIb variant of strain FA1090, a strain unrelated to strain F62. This prompted us to screen known anti-FA1090 MAbs against strain F62 PII variants, using the whole-cell assay. MAb 10.1, originally shown to be specific for PIIb and PIId of strain FA1090 (1) (Table 1) and later shown to bind in the hypervariable-2 (HV2) region of PII (6), had the identical specificity as MAb 53C4. Similarly, MAb H164, originally described as an anti-FA1090 PIIe MAb (1) (Table 1), bound F62 PII2, but not other F62 PIIs (Table 3). H164 binds to HV2 of FA1090 PIIe (Terry Connell, personal communication). H164 also recognized F62 PII2 in Western blots (data not shown; manuscript in preparation). Thus, HV2 region epitopes are shared not only within a strain (1), but also between unrelated strains.

Western blot analysis of outer membranes of several PII variants (Fig. 4) supports our whole-cell binding assays (Table 3); MAbs 53C4 and 10.1 reacted only with variants F62 PII4 and FA1090 PIIb and PIId. This suggests that not only 10.1 (1) but also 53C4 binds in the HV2 region of PII.

Effects of MAb 53C4 on gonococcus-neutrophil interactions. After identifying reactive PII variants for MAb 53C4, we examined its effects on gonococcus-neutrophil interactions. A 1:100 dilution of 53C4 inhibited peak neutrophil CL induced by FA1090 PIIb by 81 to 89% (Table 4). This is similar to previously published values obtained with MAb 10.1 (anti-FA1090 PIIb) ascites (25). In addition to being much lower than control CL values, peak CL was 75 min later than CL peaks induced by FA1090 PIIb in the absence of 53C4. MAb 53C4 displayed only moderate activity against F62 PII4-induced neutrophil CL.

We next used MAb 53C4 to examine the role of PII in adherence of gonococci to human neutrophils. Data shown for CL in Table 4 and for adherence in Table 5, experiment 1, are from the same experiment because we wished to compare the activity of the MAbs, using the same donor neutrophils with the various PII variants. The results in Table 5, experiment 2, are compiled from experiments done on two additional days. Variant F62 PII2, which does not bind MAb 53C4 (Table 3 and Fig. 4), was used as a negative control to exclude the possibility that non-immunoglobulin ascites components affected gonococcal adherence to neutrophils. MAb 53C4 specifically inhibited adherence of homologous PII variants FA1090 PIIb and F62 PII4 to neutrophils, whereas it did not inhibit adherence of heterologous variant F62 PII2 (Table 5).

TABLE 4. Inhibition by MAb 53C4 of gonococcus-induced neutrophil CL[a]

MAb	MAb titer	Peak CL, cpm (10^3) (%)		
		FA1090 PIIb	F62 PII4	F62 PII2
Expt 1				
None		335 (100)	304 (100)	164 (100)
53C4	100	65 (19)	210 (69)	154 (93)
	1,000	222 (66)	288 (95)	ND
	10,000	265 (79)	312 (103)	ND
Expt 2				
None		234 (100)	694 (100)	507 (100)
53C4	100	26 (11)	ND	ND
	1,000	128 (55)	687 (99)	ND
	10,000	231 (99)	729 (105)	ND

[a] Gonococci were incubated with MAbs at room temperature for 15 min and then used to stimulate neutrophil CL as described in Materials and Methods. Values in parentheses indicate percentage of controls with no MAbs. Results for experiments with MAbs are the means of duplicates, and the control values are means of four determinations. ND, Not determined.

DISCUSSION

To confirm the role of outer membrane protein PII in gonococcal interactions with human neutrophils, we studied the ability of previously described MAbs to inhibit nonpiliated PII$^+$ gonococci from inducing the oxidative burst of neutrophils and from adhering to neutrophils. In screening a bank of 95 antigonococcal MAbs, we found only 2 that inhibited gonococcus-neutrophil interactions, and those two reacted with PII. We consider this significant since these MAbs were raised against whole gonococci and presumably were directed against a wide variety of immunogenic gonococcal surface components. Like all other PII MAbs previ-

TABLE 5. Inhibition by MAb 53C4 of gonococcal adherence to neutrophils[a]

Variant and MAb	No. of PMNs with given no. of GC					GC per 100 PMN (%)
	0	1–2	3–5	6–8	>8	
Expt 1						
FA1090 PIIb						
No MAb	53	24	11	5	7	199 (100)
53C4	80	18	0	1	1	46 (23)
F62 PII4						
No MAb	10	28	25	15	22	505 (100)
53C4	50	26	15	4	5	187 (37)
F62 PII2						
No MAb	28	25	26	6	15	363 (100)
53C4	26	28	28	8	10	330 (91)
Expt 2						
FA1090 PIIb						
No MAb	32	23	25	9	11	329 (100)
53C4	64	19	9	0	8	160 (49)
F62 PII4						
No MAb	13	17	21	16	33	617 (100)
53C4	60	20	11	6	3	152 (25)

[a] Gonococci (GC) were incubated with the appropriate MAb (1:100 dilution) for 15 min at room temperature, added to neutrophils (PMNs), and tumbled (ratio, 50:1, gonococci/neutrophils) at 37°C for 30 min. A sample was then removed, cytocentrifuged, Wright stained, and counted to quantitate adherence.

ously described, MAbs 7VA2 and 7B9 recognized a specific PII, in this case, F62 PII3. When initiating these studies, we expected that we would find inhibition of CL or adherence by some of M. R. Tam's 95 MAbs simply on the basis of steric hindrance. For example, many of these MAbs are known to be directed against the most abundant outer membrane protein, PI (15, 38), yet they failed to inhibit gonococcus-neutrophil interactions on the basis of steric effects. The above information indicates that PII is the only immunogenic gonococcal surface component involved in adherence of nonpiliated gonococci to human neutrophils.

A previously described anti-PII MAb, 53C4 (23), significantly inhibited the interaction of gonococci with neutrophils. In addition, it bound to both F62 PII4 and FA1090 PIIb variants and inhibited their adherence to neutrophils. This is the first report of a MAb inhibiting adherence of PII^+ gonococci to human neutrophils.

We developed a rapid MAb whole-cell binding assay to determine the presence or absence of a particular PII. The assay took <1 h to perform and was found to be faster and more convenient than ELISA (22). In addition, gonococci were not exposed to denaturing agents (glutaraldehyde or detergents) prior to assay and were thus maintained in a more "native" form. This assay, along with small-volume outer membrane preparations analyzed on SDS-PAGE and colonial morphology, was instrumental in consistently documenting particular PII variants.

Western blots developed with MAb 53C4 confirmed the whole-cell binding results. The apparent identical specificity of two MAbs (10.1 and 53C4) raised against two different gonococcal strains (FA1090 and F62, respectively) containing different PIIs demonstrated that certain surface-exposed variable epitopes of PII are shared among diverse strains.

Nucleotide sequences of FA1090 PIIa, PIIb, and PIId indicate highly conserved amino acid sequences except for two hypervariable domains, HV1 and HV2 (6). If we assume that there is a common neutrophil receptor-binding site on all PIIs, then a conserved surface-exposed PII domain would be the logical choice for such a site. Thus, the MAbs studied here are apparently binding to variable surface-exposed PII epitopes near to, but not in, the receptor-binding domain of PII. If anti-PII MAbs to such a receptor-binding domain existed, they would block adherence of all PII^+ gonococci to neutrophils, yet such MAbs have not been described. Indeed, such MAbs may not be available since the PII receptor-binding domain may be physically inaccessible to antibodies. Precedence for such a hypothesis has been shown for at least two human pathogens: the human rhinovirus type 14 (28) and the human immunodeficiency virus (18).

In a previous study of MAb 10.1 ascites fluid, Rest et al. showed inhibition of gonococcus-induced CL against the homologous PII variant FA1090 PIIb (25). Virji and Heckels confirmed the role of PII in activation of neutrophils, using $F(ab)_2$ fragments of MAbs, whereas a role for pili could not be shown in such interactions (42). Adherence was not described in either of the above reports. Sugasawara et al. used very high amounts of 10.1 ascites fluid to inhibit the binding of FA1090 PIIb to HeLa cells by about 50% (31). In addition, they found relatively small differences in adherence of PII^+ and PII^- gonococci to HeLa cells. We find much larger differences between PII^+ and PII^- gonococci in their adherence to neutrophils and subsequent induction of CL (9, 24), suggesting differences in PII-mediated adherence to neutrophils and epithelial cells.

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