Lacto- and Ganglio-series Glycolipids Are Adhesion Receptors for Neisseria gonorrhoeae*

(Received for publication, April 12, 1990)

Carolyn D. Deal‡ and Howard C. Kirwan§

From the ‡Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20307, the §Laboratory of Structural Biology, National Institutes of Health, Bethesda, Maryland 20892, and ¶BioCarb Inc., Gaithersburg, Maryland 20879

The role of glycolipids as adhesion receptors for Neisseria gonorrhoeae is examined. Serum-resistant isolates, piliated and nonpiliated isogenic variants, as well as gonococci deficient in lipooligosaccharide and protein II, bind specifically to terminal and internal GlcNAcβ1-3Galβ1-4Glc and Galβ1-4Glcβ1-3Galβ1-4Glc sequences in lacto- and ganglio-series glycolipids, respectively, as measured by overlaying glycolipid chromatograms with 125I-labeled organisms. The binding activity was not affected by changing the growth conditions of the organism, as the gonococci bound to both classes of glycolipids when grown anaerobically, microaerophilically on agar or in broth, or under iron-limited conditions. The gonococci do not bind to lactosylceramide (Galβ1-4Glcβ1-1Cer) derived from lacto-N-tetraosylceramide or from asialo-GM1 by treatment with N-acetyl-β-hexosaminidase, or to other neutral glycolipids tested. Although N. gonorrhoeae bound weakly to all gangiosides on thin-layer chromatograms, including sialylparagloboside and GM3, in solid phase assays the gonococci bound with high avidity to the sequence GalNAcβ1-4Galβ1-4Glc, with moderate avidity to the sequence GlcNAcβ1-3Galβ1-4Glc, and not at all to gangliosides. Interestingly, the 4.8-kDa component of gonococcal lipooligosaccharide, which contains lacto-N-neotetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glc), strongly inhibits gonococcal-specific agglutination of human erythrocytes and inhibits the binding of labeled organisms to human paragloboside and lacto-N-tetraosylceramide on thin-layer chromatograms. Possibly, this binding specificity explains why gonococci autoagglutinate in vitro.

Gonorrhea is the most commonly reported infectious disease in the United States with as many as 2 million reported cases occurring each year (1). The organism responsible for this disease is Neisseria gonorrhoeae, an obligate human pathogen that infects diverse sites within its host including the mucous membranes of the genitourinary tract, rectum, oropharynx, and conjunctiva, although under certain conditions bacteremia and disseminated gonococcal infection also occur (2). Gonococcal infection begins with bacterial adhesion which is host-specific (3–5), suggesting that specific eucaryotic cell receptors are involved. Many studies indicate the importance of pili as adhesins mediating this process (6–13), although several different gonococcal outer membrane proteins (proteins I and II) have also been proposed to be important in adhesion (14–19). Recently, Shaw and Falkow (20) demonstrated that piliated (P') gonococci adhered in greater numbers to human endometrial cultured cells than nonpiliated (P-) gonococci; however, once bound, both were internalized with equal efficiency.

There is substantial evidence that the eucaryotic cell receptors for N. gonorrhoeae contain carbohydrate (8, 12, 21, 22), and recently it has been shown that both pilated (P') and nonpiliated (P-) gonococci bind to glycolipids (22). We report here that both lacto- and ganglio-series glycolipids containing the minimum carbohydrate sequences GlcNAcβ1-3Galβ1-4Glc and Galβ1-4Glcβ1-3Galβ1-4Glc, respectively, can support adhesion of N. gonorrhoeae and that binding to both structures is not dependent on pili, protein II (PII), or the presence of lipooligosaccharide (LOS). Interestingly, lacto-series structures are also found in some LOS derived from the outer membrane of N. gonorrhoeae (19), which may explain the well known phenomenon of autoagglutination seen with this organism.

EXPERIMENTAL PROCEDURES

Materials—Gonococcal LOS was isolated from acetone-powdered organisms by the hot phenol method (23, 24). Murine IgG monoclonal antibody 4E12 against meningococcal LOS serotype-3,7,9 (25) was obtained from Dr. Wendell Zollinger (Walter Reed Army Institute of Research, Washington, D.C.). Affinity-purified goat anti-mouse IgM (Kirkegaard and Perry, Gaithersburg, MD) was labeled with 125I (ICN Biomedicals, Costa Mesa, CA) by the IODO-GEN method (26) to a specific activity of approximately 25 μCi/μg. Bovine tests β-galactosidase, neuraminidase (Arthrobacter ureafaciens), and bovine serum albumin (BSA, fraction V) were purchased from Boehringer Mannheim. N-Acetyl-β-D-hexosaminidase was from Genzyme (Boston, MA). All standard ganglioside and neutral glycolipids were from Kirkegaard and Perry (Gaithersburg, MD). Lacto- and Ganglio-series glycolipids were from BioCarb Chemicals (Lund, Sweden). Aluminum-backed silica gel high performance thin-layer chromatography plates were purchased from Merck (West Germany). Gangliotetraosylceramide (asialo-GM2) and gangliotetraosylceramide (asialo-GD2) were prepared as described (27), α-2,3-Sialyllactosetetraosylceramide (sialylparagloboside) was isolated from type O human erythrocytes (28). Lacto-N-neotetraosylceramide (paragloboside) was desialylated by desialylylylcosidase digestion of paragloboside with α-2,3-Sialyllactosetetraosylceramide (sialylparagloboside) was isolated from type O human erythrocytes (28). Lacto-N-neotetraosylceramide (paragloboside) was desialylated by desialylylcosidase (27). The concentrations of glycolipids listed in Table I were determined by densitometry (Quick-scan, Helena Laboratories) of orcinol-stained thin-layer chromatograms compared with authentic standards. The purity of all lipids was confirmed by thin layer chromatography in neutral and acidic solvent systems.

Growth and Labeling of N. gonorrhoeae—The gonococcal strains and variants are described in Table II. The bacteria were grown in supplemented GCB (Difco) broth with shaking at 1500 rpm or on agar plates at 37 °C in 5% CO2, 95% air. Cells grown anaerobically on GCB agar were supplemented with nitrate, as described (29). Gonococci were grown under iron-limited conditions by passing the cells three times on media supplemented with 25 μM Desferal (Ciba-Geigy), as described (30). Bacteria were scraped from agar plates or harvested from broth by centrifugation and suspended in 0.01 M

*The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**The abbreviations used are: P, protein; LOS, lipooligosaccharide; BSA, bovine serum albumin; PBS, phosphate-buffered saline; HBSS, Hank’s balanced salt solution.
### TABLE I

| Glycolipid      | Structure                          | Binding |
|-----------------|------------------------------------|---------|
| Asialo-GM₁      | GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer + +    |         |
| Asialo-GM₂      | Galβ₁-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ++ +    |
| Lactotriaosylcer| GlcNAcβ₁-3Galβ₁-4Glcβ₁-1Cer +     |         |
| Paragloboside   | Galβ₁-4GlcNAcβ₁-3Galβ₁-4Glcβ₁-1Cer | +       |
| Glucocelecer (CMH)| Galβ₁-4Glcβ₁-1Cer -           |         |
| Lactosylercer (CDH)| Galβ₁-4Glcβ₁-1Cer -         |         |
| Trihexosylercer (CTH)| GalNAcβ₁-3Galβ₁-4Glcβ₁-1Cer - |         |
| Globoside (GL₁)| NeuAcα₂-3Galβ₁-4Glcβ₁-1Cer -      |         |
| Forssman (Fors)| NeuAcα₂-3Galβ₁-4Glcβ₁-1Cer -      |         |
| GU₃              | NeuAcα₂-3Galβ₁-4Glcβ₁-1Cer -      |         |
| GM₁             | NeuAcα₂-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ±       |
| GM₂             | NeuAcα₂-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ±       |
| GM₃             | NeuAcα₂-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ±       |
| Sialylparagloboside| NeuAcα₂-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ±       |
| 2,3GM₄          | NeuAcα₂-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ±       |
| 2,4GM₅          | NeuAcα₂-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ±       |
| Sialylparagloboside| NeuAcα₂-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ±       |
| ORIGIN          | NeuAcα₂-3GalNAcβ₁-4Galβ₁-4Glcβ₁-1Cer | ±       |

*Trivial names and structures are presented according to recommendations in Ref. 46 and references cited therein; cer, ceramide; CMH, ceramide monohexoside; CDH, ceramide dihexoside (lactosylceramide); CTH, ceramide trihexoside; GL₁, globoside.

-Negative binding (−) indicates no binding to 2 μg of glycolipid and positive binding to less than 0.4 μg (+ +), 0.8–1 μg (+), and 1 μg (+).*

### TABLE II

| Strain (Ref.) | Phenotype   | Binding to Asialo-GM₁ | Binding to Paragloboside |
|---------------|-------------|-----------------------|--------------------------|
| MS₁₁₁ (42)    | P*, PI⁻⁻⁻   | ++ +                  | +                        |
| MS₁₁₃ (42)    | P*, PI⁻⁻⁻   | ++ +                  | +                        |
| FA5100 (43)   | LOS/MAb un-reactive | ++ +                  | Serum resistant +         |
| WG-52 (44, 45)|             |                       |                          |

*Abbreviations used: P*, pilated; P*, nonpiliated; PI⁻⁻⁻, protein II deficient; LOS, lipooligosaccharide deficient; MAb, monoclonal antibody.*

---

**Fig. 1. Binding of ^125^I-labeled N. gonorrhoeae strain MS₁₁₁ (P*, PI⁻⁻⁻) to paragloboside and lacto-N-triaosylceramide separated by thin-layer chromatography.** Glycophobes were chromatographed on aluminum-backed silica gel high performance thin-layer chromatography plates developed in chloroform, methanol, 0.25% KCl in water, 5:4:1. The plates were coated with 0.1% polyisobutylmethacrylate, soaked in Tris-buffered saline-BSA, and incubated for 2 h at 25 °C with ^125^I-labeled gonococci suspended in HBSS-BSA (panel B) or sprayed with orcinol reagent to identify glycolipids (panel A). Lanes 1, 1 μg each of galactosylceramide (CMH), lactosylceramide doublet (CDH), trihexosylceramide (CTH), globoside (GL₁), Forssman glycolipid (FORS), and gangliosides GM₁, GM₂, GM₃, GD₃, GD₄, GD₅, and GD₆; lanes 2, 1 μg of sialylparagloboside; lanes 3, 1 μg of paragloboside derived from sialylparagloboside by treatment with neuraminidase; lanes 4, lacto-N-triaosylceramide derived from paragloboside by treatment with β-galactosidase; lanes 5, 1 μg of lactosylceramide derived from lacto-N-triaosylceramide by treatment with N-acetyl-β-hexosaminidase. For structures see Table I.

---

**RESULTS AND DISCUSSION**

**Binding of N. gonorrhoeae to Lacto-series Glycolipids on Chromatograms**—The glycolipid binding specificity of the different gonococcal isogenic variants was established by the thin-layer overlay assay and is summarized in Table II. As shown by an autoradiogram (Fig. 1B) compared with an identical thin-layer plate visualized with orcinol reagent (Fig. 1A), N. gonorrhoeae binds avidly to authentic paragloboside and the product obtained after β-galactosidase treatment, lacto-N-triaosylceramide (Fig. 1B, lanes 3 and 4). No binding was detected to lactosylceramide derived from lacto-N-triaosylceramide after treatment with N-acetyl-β-hexosaminidase (Fig. 1B, lane 5) or to the other neutral glycolipids tested (Fig. 1B, lane 1; Table I). These data appear to be in variance with those of Stromberg et al. (22) who reported that N. gonorrhoeae does not bind to lacto-N-triaosylceramide and lacto-
N. gonorrhoeae Adhesion to Glycolipids

Fig. 2. Binding of 125I-labeled N. gonorrhoeae strain MS11_{as} (P^{-}, PII^{-}) to asialo-G_{M_1} and asialo-G_{M_2} separated by thin-layer chromatography. Panel A, glycolipids detected by orcinol reagent; panel B, autoradiogram of chromatogram overlaid with radiolabeled gonococci as described in the legend of Fig. 1. Lanes 1, 1 μg of G_{M_1}; lanes 2, 1 μg of asialo-G_{M_1} derived from G_{M_1} by treatment with neuraminidase; lanes 3, 1 μg of asialo-G_{M_2} derived from asialo-G_{M_2} by treatment with β-galactosidase; lanes 4, 2 μg of lactosylceramide derived from asialo-G_{M_2} by treatment with β-N-acetyhexosaminidase. For structures see Table I.

Fig. 3. Binding of N. gonorrhoeae strain MS11_{as} (P^{-}, PII^{-}) to immobilized glycolipids. Lipids in 25 μl of methanol containing 0.1 μg each of the auxiliary lipids cholesterol and phosphatidylcholine were evaporated in flat-bottom wells of polyvinylchloride microtiter plates. The wells were blocked with 1% albumin overnight at 4 °C, washed twice with HBSS-BSA, and incubated at 23 °C with 25 μl of 125I-labeled N. gonorrhoeae (approximately 10^6 cpm). After 2 h, the wells were washed 5 times with saline, cut from the plate, and bound radioactivity was quantified in a scintillation counter. In control experiments gonococci were incubated with auxiliary lipids only to correct for nonspecific binding (typically <1% of the total radioactivity added). N. gonorrhoeae binding was determined in HBSS-BSA for asialo-G_{M_2} (■), paragloboside (▲), and lactosylceramide, G_{M_1}, or sialylparagloboside (□).

N-neotetraosylceramide (paragloboside). Possibly, this discrepancy is related to the source and/or fatty acid contents (22) of the glycolipids used by these investigators. In some experiments, N. gonorrhoeae bound weakly to some of the gangliosides (Table I) including sialylparagloboside (Fig. 1B, lane 2) and G_{M_1} (Fig. 1B, lane 1); however, this observation was not always reproducible (Fig. 2B, lane 1), and none of the gangliosides demonstrated concentration-dependent binding in microtiter plates (Fig. 3).

Binding of N. gonorrhoeae to Purified Ganglio-series Glycolipids on Chromatograms—In agreement with the findings of Stromberg et al. (22), P^{-} and PII^{-} variants of N. gonorrhoeae, bind to gangliotetraosylceramide (asialo-G_{M_2}) and gangliotriaosylceramide (asialo-G_{M_2}) (Fig. 2). The organism did not bind to lactosylceramide derived from asialo-G_{M_2} by treatment with β-galactosidase (Fig. 2B, lane 4) suggesting that the β1-4-linked GalNAc, which is positioned internally in asialo-G_{M_2} and terminally in asialo-G_{M_3}, is required for binding (Table I). As has been shown for many pulmonary pathogenic bacteria, including Pseudomonas aeruginosa, Haemophilus influenzae, and Streptococcus pneumoniae (27), all of the variants of N. gonorrhoeae tested (Table II) preferred GalNAcβ1-4Galβ1-4Glc sequences unsubstituted with sialyl residues for binding (Fig. 2B, lane 1). Interestingly, P. aeruginosa, H. influenzae, and S. pneumoniae also bind avidly to paragloboside. Binding patterns similar to those shown in Figs. 1B and 2B were also observed when the plates were incubated with all other gonococcal variants used in this study (Table II).

Quantitative Binding of N. gonorrhoeae to Immobilized Glycolipids and Inhibition of Binding by Glycolipids—Binding of N. gonorrhoeae to purified glycolipids adsorbed on microtiter plates was examined to further define the binding specificity and to compare the relative avidities of the lacto- and ganglio-series receptors. As shown in Fig. 3, the gonococci bind better to asialo-G_{M_2} than to paragloboside and not at all to sialylparagloboside, G_{M_1}, or lactosylceramide. Binding to both asialo-G_{M_1} (data not shown) and asialo-G_{M_2} is half-maximal at 0.2 μg, 7 times better than to paragloboside (or lacto-N-triaosylceramide, data not shown), suggesting a higher avidity for the ganglio-series glycolipids. Binding of P^{-} and PII^{-} variants of N. gonorrhoeae to both asialo-G_{M_2} and paragloboside was not affected by changing the growth conditions of the organism, as the gonococci bound equally well to both glycolipids when grown anaerobically, microaerophilically on agar or in broth, or under iron limited conditions (data not shown).

Biological Significance—N. gonorrhoeae agglutinate human erythrocytes (33) and adhere to and are phagocytosed by neutrophils (34). The receptor that mediates binding of these cells to the gonococci is probably paragloboside and lacto-N-triaosylceramide which are present in substantial amounts in both cell types (23, 27, 35, 36). Lacto-series also constitute the major glycolipid component of various tissues and organs and are glycolipid precursors of the major blood group antigens. Interestingly, Galα1-4GlcNAcβ1-3Galβ1-4Glc ... sequences are also present in many gonococcal LOS (13). As shown in Fig. 4, an anti-LOS antibody (4BE12) (25) binds strongly to authentic human paragloboside, detecting as little as 50 ng of glycolipid. Gonococcal lipopolysaccharide which contains the 4.8-kDa LOS component is bound by monoclonal antibody 4BE12 thus confirming the presence of the Galβ1-4GlcNAcβ1-3Galβ1-4Glc ... sequence in this lipopolysaccharide (data not shown). This LOS also strongly inhibits gonococcal specific agglutination of human erythrocytes as well as inhibits the binding of radiolabeled P^{-} and PII^{-} gonococci to paragloboside and lacto-N-triaosylceramide on thin-layer chromatograms (data not shown). Thus, the mechanism for the well known phenomenon of gonococcal autoagglutination (37, 38) may be explained by an adhesin of one organism binding to GlcNAcβ1-3Galβ1-4Glc sequences in the LOS of another organism. As bacterial LOS do not contain ceramide,
binding of \textit{N. gonorrhoeae} to at least the lacto-series glycolipids is probably not dependent on the fatty acid in ceramide as has been reported for other bacteria (39).

Asialo-GM\textsubscript{2} has been reported to occur in cultured human endocervical cells (22), a relevant target tissue for infection, and asialo-gangliosides also occur in other human tissues, although in lower amounts (27, 40, 41). Both asialo-GM\textsubscript{1} and asialo-gangliosides also occur in other human tissues, and asialo-GM\textsubscript{1} has been reported to occur in cultured human endocervical cells (22), a relevant target tissue for infection.

FIG. 4. Binding of anti-LOS monoclonal antibody 4BE12 to authentic paragloboside. Panel A, glycolipids detected by orcinol staining; panel B, autoradiogram of chromatogram protected by orcinol staining; panel II, lactosylceramide clonal antibody 4BE12 to authentic paragloboside. Lanes d, 1, 2, 3, 4, 5, 6, 7, 8, and 9 are 0.5, 0.25, 0.125, 0.062, 0.031, and 0.016 \mu g of paragloboside, respectively.

REFERENCES

1. Center for Disease Control (1988) Morbid. Mortal. Weekly Rep. 36, 54.
2. Hook, E. W., III, and Holmes, K. K. (1988) Ann. Intern. Med. 109, 229-243.
3. Johnson, A. P., Taylor-Robinson, D., and McGee, Z. A. (1977) Infect. Immun. 18, 833-839.
4. McGee, Z. A., Johnson, A. P., and Taylor-Robinson, D. (1975) Infect. Immun. 13, 608-618.
5. Johnson, A. P., Clark, J. B., Oberm, M. F., and Taylor-Robinson, D. (1980) Br J. Exp. Pathol. 61, 521-527.
6. McGee, Z. A., Johnson, A. P., and Taylor-Robinson, D. (1981) J. Infect. Dis. 143, 413-422.
7. Trus, T. J., Lambden, P. R., and Watt, P. J. (1980) J. Gen. Microbiol. 119, 179-187.
8. Pearce, W. A., and Buchanan, T. M. (1978) J. Clin. Invest. 61, 931-943.
9. Swanson, J. (1973) J. Exp. Med. 137, 571-588.
10. Ofek, I., Beachey, E. H., and Biss8, A. L. (1974) J. Infect. Dis. 129, 316-318.
11. Mar, L. L., Stroogen, R. A., and Davies, K. J. (1988) Infect. Immun. 56, 1743-1777.
12. Gubish, E. R., Jr., Chen, K. C. S., and Buchanan, T. M. (1982) Infect. Immun. 37, 189-194.
13. Mandrell, R. E., Grattis, J. M., and Macher, B. A. (1988) J. Exp. Med. 168, 107-129.
14. Besen, D., and Gotschlich, E. C. (1986) Infect. Immun. 54, 154-160.
15. Lambeth, P. R., Heckels, J. E., James, L. T., and Watt, P. J. (1979) J. Gen. Microbiol. 114, 305-312.
16. Rest, R. F., Lee, N., and Bowden, C. (1988) Infect. Immun. 50, 116-122.
17. Vrij, M., and Heckels, J. E. (1986) J. Gen. Microbiol. 132, 503-512.
18. Fischer, S. H., and Rest, R. F. (1986) Infect. Immun. 56, 1374-1379.
19. Lev-Schmidt, G., Schmitt, S., and Buchanan, T. M. (1989) Int. J. Med. Microbiol. 271, 158-170.
20. Shaw, J. H., and Fallows, S. (1988) Infect. Immun. 56, 1625-1632.
21. Schoolnik, G. K., Fernandez, R., Tai, J. Y., Rothbard, J., and Gotschlich, E. C. (1984) J. Exp. Med. 159, 1351-1370.
22. Stromberg, N., Dein, C., Nyberg, C., Normark, S., So, M., and Karlsson, K.-A. (1986) Proc. Natl. Acad. Sci. U. S. A. 85, 4992-4996.
23. Bertram, M. A., Grattis, J. M., and Broad, D. D. (1976) J. Infect. Med. 116, 842-846.
24. Westphal, O., and Jann, K. (1965) in Methods in Carbohydrate Chemistry (Whistler, R. L., ed) pp. 83-91, Academic Press, New York.
25. Zollinger, W. D., and Mandrell, R. E. (1980) Infect. Immun. 29, 451-458.
26. Fraker, P. J., and Speck, J. C. (1978) Biochem. Biophys. Res. Commun. 80, 849-857.
27. Kriyan, H. C., Roberts, D. D., and Ginsburg, V. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6157-6161.
28. Ando, S., Kon, K., Irobe, M., Nagai, Y., and Yamakawa, T. (1976) J. Biochem. (Tokyo) 79, 625-632.
29. Knapp, J. S., and Clark, V. L. (1984) Infect. Immun. 46, 176-181.
30. Micheelson, P. A., Blackman, E., and Sparling, P. F. (1982) Infect. Immun. 35, 915-920.
31. Kriyan, H. C., Ginsburg, V., and Roberts, D. D. (1988) Arch. Biochem. Biophys. 260, 493-496.
32. Micheelson, P. A., Spitalnik, S. L., and Ginsburg, V. (1987) Methods Enzymol. 159, 195-207.
33. Wissman, G. M., Mcnicol, P., Lian, C. J., and Primrose, D. S. (1981) Can. J. Microbiol. 27, 1053-1061.
34. Shafer, W. M., and Rest, R. F. (1989) Annu. Rev. Microbiol. 43, 121-145.
35. Macher, B. A., and Klock, J. C. (1980) Biochem. Biophys. Res. Commun. 90, 147-155.
36. Swanson, J. L., Krauss, S. J., and Gotschlich, E. C. (1971) J. Exp. Med. 134, 884-895.
37. Swanson, J. L. (1978) Infect. Immun. 19, 320-321.
38. Stromberg, N., Fod, M., Lindberg, A., and Karlsson, K.-A. (1988) FEBS Lett. 232, 193-198.
39. Spitalnik, P. F., Darley, J. M., Burger, S. R., and Spitalnik, S. L. (1989) Arch. Biochem. Biophys. 278, 576-591.
40. Gillard, B. K., Jones, M. A., and Marcus, D. M. (1987) Arch. Biochem. Biophys. 256, 435-445.
41. Parachuri, D. K., Seifert, H. S., Ajioka, R. S., Karlsson, K.-A., and So, M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 352-357.
42. Swanson, J. L., Barrera, O., Sola, J., and Boslig, J. (1988) J. Exp. Med. 168, 2121-2129.
43. Shafer, W. M., Joiner, K., Guymon, L. F., Cohen, M. S., and Sparling, P. F. (1984) J. Infect. Dis. 149, 175-183.
44. Rice, P. A., and Kasper, D. L. (1982) J. Clin. Invest. 70, 157-167.
45. UFPAC-IB Joint Commission on Biochemical Nomenclature (1986) Eur. J. Biochem. 158, 1-6.