purification protocols. Isolation of full length cDNA for the glucocorticoid receptor with the immunopositive clone DNA's as probes will allow determination of its primary amino acid structure.

CARY WEINBERGER
STANLEY M. HOLLENBERG*
ESTELITA S. ONG
*Salk Institute for Biological Studies, San Diego, California 92138*
JEFFREY M. HARMON
*Uniformed Services University of the Health Sciences, Bethesda, Maryland 28814*
STEVEN T. BROWER
*Boston University School of Medicine, Boston, Massachusetts 02115*
JOHN CIDLOWSKI
*University of North Carolina, Chapel Hill 27514*
E. BRAD THOMPSON
*University of Texas Medical School, Galveston 77550*
MICHAEL G. ROSENFELD
*School of Medicine, University of California, San Diego, La Jolla 92093*
RONALD M. EVANS
*Salk Institute for Biological Studies*

### References and Notes

1. J. Gorski and F. Gannon, *Annu. Rev. Physiol.* **38**, 425 (1976); J. D. Baxter and P. H. Forsham, *Am. J. Med.* **53**, 573 (1972).
2. E. V. Jensen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 632 (1968); K. R. Yamamoto and B. M. Alberts, *Annu. Rev. Biochem.* **45**, 721 (1976); M. R. Sherman and J. Stevens, *Annu. Rev. Physiol.* **46**, 83 (1984).
3. G. M. Ringold, K. R. Yamamoto, J. M. Bishop, H. E. Varmus, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2879 (1977); K. E. Mayo and R. D. Palmiter, *J. Biol. Chem.* **256**, 2621 (1981); S. R. Spindler, S. H. Mellon, J. D. Baxter, *ibid.* **257**, 11627 (1982); R. E. Evans, N. C. Birnberg, M. G. Rosenfeld, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7659 (1982).
4. F. Payvar, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6628 (1981); M. Pfahl, *Cell* **31**, 475 (1982).
5. N. Hynes *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3637 (1983); M. Karin *et al.*, *Nature (London)* **308**, 513 (1984).
6. J. Carlstedt-Duke, S. Okret, O. Wrange, J.-A. Gustafsson, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4260 (1982); M. Pfahl, R. Ketheher, S. Bourgeois, *Mol. Cell. Endocrinol.* **10**, 193 (1978); H. G. Dellweg, A. Hotz, K. Mugele, U. Gehring, *EMBO J.* **1**, 285 (1982).
7. S. Okret, J. Carlstedt-Duke, O. Wrange, K. Carlstrom, J.-A. Gustafsson, *Biochim. Biophys. Acta* 677(2), 205 (1981); H. Westphal, G. Moldenhauer, M. Beato, *EMBO J.* **1**, 1467 (1982).
8. J. M. Harmon *et al.*, *Cancer Res.* **44**, 4540 (1984).
9. O. Wrange, J. Carlstedt-Duke, J. Gustafsson, *J. Biol. Chem.* **254**, 9284 (1979).
10. S. Simons and E. B. Thompson, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3541 (1981); U. Gehring and A. Hotz, *Biochemistry* **22**, 4013 (1983).
11. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
12. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
13. Rat liver cytosol was prepared from 18 adrenalectomized rats and labeled with $^3$H-triamcinolone acetonide (*8, 9*). Labeled receptor was fractionated twice on DEAE cellulose with a KCl gradient. Fractions containing unactivated receptor eluted from the first column at KCl concentrations between 150 m*M* and 200 m*M*. These fractions were precipitated with ammonium sulfate (40 percent saturation) to activate the receptor and then passed through a second DEAE cellulose column in 100 m*M* KCl. Analysis of the column flow-through by Western blotting with crude antiserum (1:200 dilution) detected only the 94-kD band. The flow-through fraction (20 mg) was covalently coupled to Affi-Gel 10 (Bio-Rad) and antibody was purified as described (*18*).
14. G. G. Rousseau, J. D. Baxter, S. H. Higgins, G. M. Tomkins, *J. Mol. Biol.* **79**, 539 (1973).
15. R. A. Young and R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1194 (1983). Cytoplasmic [poly(A)$^+$] RNA was isolated from IM-9 cells and fractionated by sedimentation through a 15 to 30 percent sucrose gradient. RNA's greater than 18*S* in size were pooled and used as templates with oligodeoxythymidylate as primer for cDNA synthesis with avian myeloblastosis virus reverse transcriptase (Bethesda Research Laboratories). Double stranded molecules (100 ng) were synthesized as described [T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982)]. Synthetic Eco RI linkers were ligated to these molecules which were then inserted into Eco RI–digested λgt11 DNA.
16. D. E. Smith and P. A. Fisher, *J. Cell. Biol.* **99**, 20 (1984).
17. Filter disks with immobilized proteins from immunopositive lysogens were prepared by overlaying on 2000 phage plaques. For epitope selection, 50 μl of crude antiserum was diluted with a solution of 0.5 percent Triton X-100 and 3 percent bovine serum albumin (BSA) in TBS (50 m*M* tris, *p*H 8.0, and 150 m*M* NaCl) and incubated with the filters for 1 hour. Filters were washed three times with a solution of 0.5 percent Triton X-100 in TBS and blotted dry. Antibody was eluted separately from each filter with three 1 minute washes in 5 m*M* glycine-HCl (*p*H 2.3), 150 m*M* NaCl, 0.5 percent Triton X-100, and BSA (100 μg/ml). The combined washes were immediately neutralized with tris-HCl *p*H 7.4 to a final concentration of 50 m*M*. These antibodies were reacted with Western blots as described (*12*).
18. R. A. Young and R. W. Davis, *Science* **222**, 778 (1983). The fusion protein synthesized by clone HGR2.9C was precipitated by addition of ammonium sulfate to 33 percent saturation. This material, containing approximately 1 percent fusion protein, was coupled to Affi-Gel 10. To purify antibody, 50 μl of crude GR 884 were diluted in TBS, 0.5 percent Triton X-100, and 3 percent BSA, and passed through the Affi-Gel column. The column was washed extensively with the dilution buffer and then with a solution of 5 m*M* tris-HCl (*p*H 7.4), 150 m*M* NaCl, and 0.5 percent Triton X-100. Bound antibody was eluted with 5 m*M* glycine-HCl (*p*H 2.3), 150 m*M* NaCl, 0.5 percent Triton X-100, and BSA (100 μg/ml) and neutralized with tris-HCl (*p*H 7.4) to a final concentration of 50 m*M*.
19. E. Southern, *J. Mol. Biol.* **98**, 503 (1975).
20. Cytoplasmic and nuclear extracts were prepared by lysing cells in 10 m*M* Hepes (*p*H 7.5), 5 m*M* $MgCl_2$, 1 m*M* $Na_2MoO_4$, 5 m*M* benzamidine, 5 m*M* iodoacetic acid, 1 m*M* phenylmethylsulfonyl fluoride and aprotinin (2.5 μg/ml). Centrifugation in a Beckman JS5.2 rotor at 2000 rev/min for 2 minutes yielded a cytoplasmic fraction (supernatant) which was further centrifuged in a Beckman Ti60 rotor at 35,000 rev/min for 30 minutes. The nuclear pellet was washed twice in lysis buffer with 0.1 percent NP-40 and chromatin-bound protein was released by addition of NaCl to 0.5*M*.
21. T. Maniatis, A. Jeffrey, G. D. Kleid, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1184 (1975).
22. We thank R. Manohar, S. Hochschwender, J. Cooper, and J. Lindstrom for helpful discussions and suggestions on Western blot analysis and protein purification, and S. Crews, J. de Wet, N. Oliver, and G. Wahl for advice on preparation of the λ library. We wish to especially thank K. Mayo for both technical advice and discussion from the inception of this work. Supported by grants from NIH, a Damon Runyon-Walter Winchell Cancer Fund Fellowship DRG-755 (C.W.), an NIH predoctoral training grant to the Department of Biology, University of California at San Diego (S.H.), and the Alexander Foundation (R.M.E.).

* S.M.H. is a postdoctoral trainee in the Department of Biology, University of California, San Diego.

20 November 1984; accepted 28 January 1985

## Expression of the Chlamydial Genus-Specific Lipopolysaccharide Epitope in *Escherichia coli*

Abstract. *The obligate intracellular prokaryote* Chlamydia trachomatis *is the etiological agent of trachoma and is a primary causative pathogen of sexually transmitted genital tract disease; both diseases affect millions of people each year. The cloning of genes encoding the enzyme or enzymes producing the genus-specific lipopolysaccharide antigen of* Chlamydia *into* Escherichia coli *is reported here. The cloned chlamydial lipopolysaccharide antigen appears to be a hybrid lipopolysaccharide molecule composed of both* Chlamydia *and* Escherichia coli *components. The chlamydial lipopolysaccharide antigen is expressed on the surfaces of the viable* Escherichia coli *recombinants. These findings may have a significant impact on defining the role of this highly conserved antigen in the pathogenesis and diagnosis of chlamydial infections.*

*Chlamydia* are obligate intracellular prokaryotes that are structurally similar to gram-negative bacteria (*1*). There are two species, *Chlamydia trachomatis* and *C. psittaci*. *Chlamydia trachomatis* is a specific human pathogen, whereas *C. psittaci* is a pathogen of a variety of nonprimate species. Diseases caused by *C. trachomatis* present a major health problem throughout the world. In developing countries, *C. trachomatis* causes hyperendemic trachoma, which is the world's leading cause of preventable blindness. There are approximately 2 million people in the world today who are blind as a result of trachoma and an estimated 360 million more who are affected by this disease (*2*). In industrialized nations, *C. trachomatis* causes an array of sexually transmitted genital tract and associated infections whose incidence is increasing at epidemic rates (*3*). Conservative estimates indicate that in the United States alone there are at least 3 million *C. trachomatis* infections per year (*3*). In addition to their vertebrate hosts, chlamydial-like organisms have also been identified in tissues of spiders *(Coelotus luctuosus)* (*4*) and clams *(Mercenaria mercenaria)* (*5*);

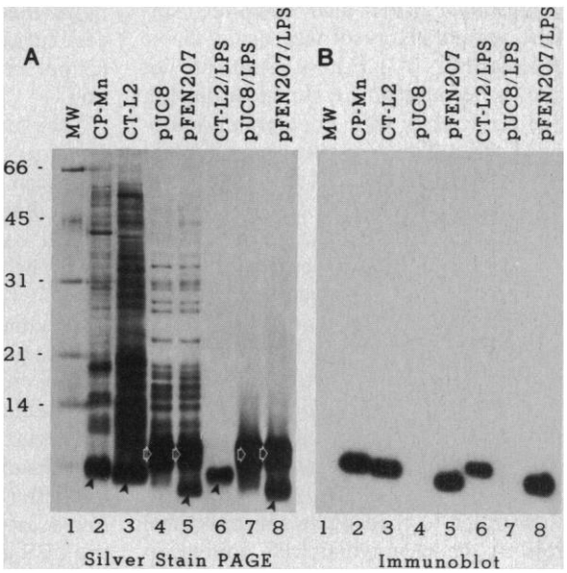


Fig. 1. Polyacrylamide gel and immunoblot analysis of recombinant *E. coli* clones expressing the genus-specific chlamydial LPS epitope. (A) Photograph of a silver-stained 15 percent polyacrylamide gel: (1) MW, molecular weight standards (in kilodaltons); (2) CP-Mn, whole-cell preparation of *C. psittaci* Cal-10 meningopneumonitis strain; (3) CT-L2, whole-cell preparation of *C. trachomatis* serotype L2; (4) pUC8, whole-cell preparation of *E. coli* JM109 harboring plasmid pUC8; (5) pFEN207, whole-cell preparation of *E. coli* JM109 harboring plasmid pFEN207; (6) CT-L2/LPS, *C. trachomatis* serotype L2 LPS isolated by the phenol-water procedure of Westphal and Jann (*20*); (7) pUC8/LPS, isolated LPS from *E. coli* JM109 with plasmid pUC8; (8) pFEN207/LPS, isolated LPS from *E. coli* JM109 with plasmid pFEN207. The two LPS species (migrating as a doublet) of *E. coli* JM109 are identified by the open arrows in lanes 4, 5, 7, and 8. The chlamydial LPS and a faster migrating LPS found only in whole cell preparations and the phenol-water extract of JM109(pFEN207) are identified by the solid arrows. This smaller molecular weight species of LPS found only in *E. coli* harboring plasmid pFEN207 was immunoreactive with monoclonal antibody against the genus-specific chlamydial LPS epitope as shown by the immunoblot. (B) Immunoblot of a polyacrylamide gel shown in (A). Polypeptides and LPS were electrophoretically transferred to nitrocellulose paper, reacted with monoclonal antibody against the genus-specific chlamydial LPS epitope, and probed with [$^{125}$I]protein A as described (*12*). The monoclonal antibody recognizes the LPS of *C. psittaci* and *C. trachomatis* (lanes 2 and 3) and the *C. trachomatis* LPS isolated by phenol-water extraction of purified chlamydial elementary bodies. The monoclonal antibody also binds to the lower molecular weight LPS in both the whole-cell preparation (lane 5) and the phenol-water extract (lane 8) of *E. coli* harboring the pFEN207 plasmid. The monoclonal antibody did not react with the whole-cell preparation or isolated LPS of *E. coli* harboring pUC8 (lanes 4 and 7). These findings show that *E. coli* (pFEN207) is expressing at least two electrophoretically different species of LPS: a larger molecular weight LPS common to *E. coli* JM109 and a smaller LPS species that binds the genus-specific chlamydial LPS monoclonal antibody.

these results suggest that chlamydiae are ubiquitous parasites present throughout the animal kingdom.

Biologically, *Chlamydia* are distinguished from all other intracellular prokaryotes by their unique life cycle and molecular mechanisms of pathogenesis. The life cycle consists of two cell types, the elementary body and the reticulate body, which differ both morphologically and functionally (*1*). The elementary body is the extracellular cell type; it is metabolically inactive and is the infectious form of the parasite. The elementary body is unique in that it is efficiently phagocytized, even by nonprofessional phagocytes (*6*), and, once internalized, it prevents phagolysosomal fusion (*7*). Mediators of these interesting pathogenic mechanisms are believed to be surface components of the elementary body; however, their identity and biochemical nature are currently unknown. Despite their common biology, *C. trachomatis* and *C. psittaci* share only 10 percent DNA homology (*8*) and demonstrate little antigenic relatedness among their surface protein constituents (*9*). They do, however, share a common outer-membrane glycolipid antigen that has recently been shown to be a lipopolysaccharide (LPS) antigenically similar to the Re LPS chemotype isolated from mutants of *Salmonella* spp. (*10–12*). Despite the similarities between chlamydial LPS and the Re LPS of enteric bacteria, chlamydial LPS contains a periodate-sensitive antigenic determinant that is not found in a variety of Gram-negative bacteria (*12*). This antigenic determinant has been defined by a monoclonal antibody and termed the genus-specific LPS antigen (*12*). The biological function of chlamydial LPS is unknown, but the conserved nature of the LPS epitope in an otherwise antigenically diverse genus suggests that this epitope may be functionally important with respect to the molecular mechanisms of pathogenesis shared by these obligate intracellular parasites.

From a gene bank (*13*) of *C. trachomatis* (strain LGV-434) DNA, we identified several recombinants that were immunoreactive with rabbit polyclonal antibody raised against viable LGV-434 elementary bodies. Four of these nonsibling recombinants were subsequently shown by immunoblots of polyacrylamide gels to possess an antigen reactive with a monoclonal antibody directed against the genus-specific LPS epitope. One of these *Escherichia coli* recombinants, JM109(pFEN207), was studied in more detail. Analysis of whole-cell preparations and phenol-water extracts of chlamydial elementary bodies and *E. coli* recombinants by polyacrylamide gel electrophoresis (PAGE) showed that the JM109(pFEN207) recombinant has three distinct species of LPS (Fig. 1A). Two of the LPS species were found in the parent *E. coli* strain harboring the pUC8 plasmid. A third LPS species was unique to the recombinant strain and reacted with a monoclonal antibody against the chlamydial genus–specific LPS epitope by immunoblotting analysis (Fig. 1B). The immunoreactive LPS found in the recombinant clone pFEN207 migrated with an increased electrophoretic mobility relative to native chlamydial LPS.

There are two likely explanations for this observation: (i) the molecule is an incomplete chlamydial LPS, or (ii) it is a hybrid LPS molecule composed of both *E. coli* and chlamydial LPS component. The latter explanation is more likely since the 6.5-kilobase chlamydial DNA insert in the pFEN207 plasmid lacks the necessary coding capacity for the estimated 20 to 30 enzymes needed to synthesize a complete LPS molecule (*14, 15*). Using this reasoning, we believe that the pFEN207 plasmid contains a gene or genes that encode a glycosyl transferase involved in chlamydial LPS synthesis and that the chlamydial glycosyl transferase has incorporated the carbohydrate moiety that confers the genus-specific antigenic property to chlamydial LPS on *E. coli* LPS. Glycosylation of the *E. coli* LPS by the putative chlamydial glycosyl transferase might terminate the further synthesis of the proximal portion of the *E. coli* LPS and result in a truncated hybrid molecule. Although this is a reasonable explanation for our observations, definitive proof will require structural and genetic analysis.

Of considerable interest to us was whether the chlamydial LPS epitope was exposed on the surface of the *E. coli* recombinant. Fluorescent antibody staining of heat-fixed pFEN207 cells with the chlamydial LPS monoclonal antibody showed that the antigen was located at the periphery of individual

cells (Fig. 2).We also observed surface immunofluorescence, using viable JM109(pFEN207). (These results are not shown because of the technical difficulties associated with photographing unfixed cells.) We were able to confirm the surface exposure of the chlamydial LPS epitope on viable *E. coli* recombinants by immunoelectron microscopy. When viable pFEN207 cells were reacted with monoclonal antibody and protein A colloidal gold and then examined by electron microscopy without subsequent fixation or staining, electron-dense gold particles were specifically bound to the surfaces of the recombinant clone expressing the chlamydial LPS epitope (Fig. 2C).

In order to investigate the biological role of the chlamydial LPS epitope in chlamydial–host cell interaction, we performed some preliminary in vitro experiments. The *E. coli* recombinant, JM109(pFEN207), and its parent strain, JM109(pUC8), were radiolabeled both intrinsically ([$^{35}$S]methionine) and extrinsically ($^{125}$I) and absorbed to monolayer cultures of HeLa 229 cells at 37°C. In both sets of experiments, we observed that the association of the recombinant strain with HeLa cells was two to five times that of the parent strain. These data suggest a possible role for the LPS epitope in chlamydial–host cell interaction.

Thus far, all the LPS biosynthetic enzyme genes that have been cloned have been from enteric bacteria (*16–18*); enzymatic activity has not always been observed outside of the homologous system. Therefore, our results are particularly interesting since they indicate similar mechanisms of LPS biogenesis in two diverse genera. Furthermore, the observations reported here will provide an opportunity to investigate the function of chlamydial LPS in both the biology of the chlamydial infectious process and the pathogenesis of chlamydial disease. For example, *E. coli* clones expressing the LPS genus-specific antigen on their surfaces may be useful in studying the role of this molecule as a chlamydial adhesion or a virulence determinant that functions in the internalization or inhibition of phagolysosome inhibition, or both. Moreover, it has been proposed that the pathology seen in chlamydial disease, particularly chronic ocular trachoma, is immunologically mediated since pathognomonic signs of pannus formation and conjunctival scarring occur only after repeated or recurrent infection (*19*). The antigen or antigens that mediate this apparent hypersensitivity response to infection are unknown.

It is tempting to speculate, however, that the antigenically common LPS determinant may mediate this proposed immunopathogenesis. The recombinant *E. coli* clone JM109(pFEN207) expressing the chlamydial genus–specific LPS epitope could be used in animal model studies to investigate the role of chlamydial LPS in both the pathogenesis and immunity to chlamydial infections. This recombinant may prove to be a useful reagent in the development of much needed immunoassays for the diagnosis of chlamydial infections in both humans and nonprimate hosts.

FRANCIS E. NANO
HARLAN D. CALDWELL
*Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, Hamilton, Montana 59840*

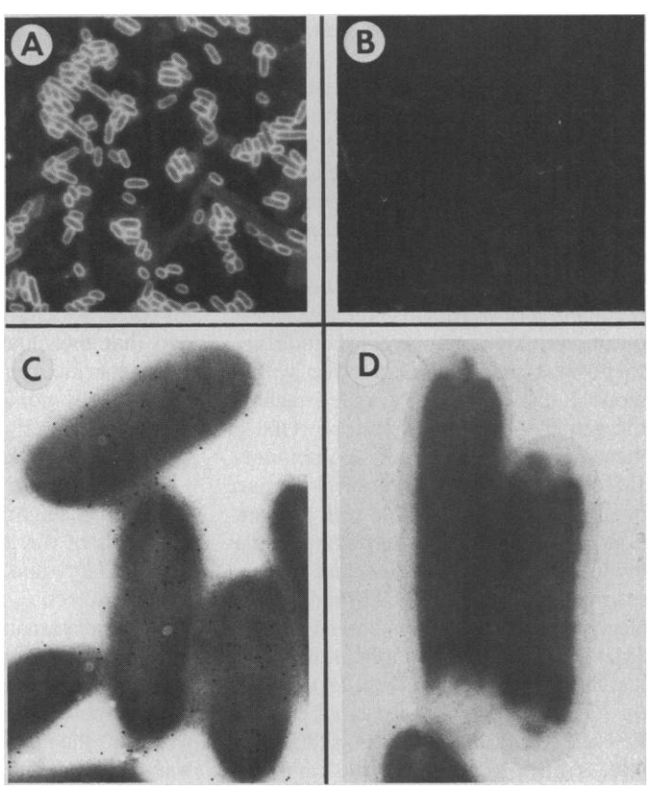


Fig. 2. Fluorescent antibody staining and immunoelectron microscopy showing the outer-membrane location and surface exposure of the genus-specific chlamydial LPS epitope expressed in *E. coli*. (A) Fluorescence photomicrograph of fixed *E. coli* strain JM109 with recombinant plasmid pFEN207 reacted with monoclonal antibody against the genus-specific chlamydial LPS epitope and stained with a fluorescein-conjugated goat antimouse immunoglobulin G antibody. Fluorescence is localized to the outer membrane of *E. coli*. (B) Negative control, *E. coli* JM109 with plasmid pUC8 treated in the same way as cells shown in (A). (C) Immunoelectron microscopy of viable *E. coli* (pFEN207) reacted with monoclonal antibody and probed with protein A conjugated to 14 n*M* colloidal gold particles (*21, 22*). The electron-dense gold particles are found associated only with the surfaces of viable *E. coli*. Binding of monoclonal antibody against the chlamydial LPS epitope shows that this antigenic determinant is exposed on the native *E. coli* cell surface. (D) Negative control, *E. coli* pUC8 treated in the same way as cells shown in (C).

**References and Notes**

1. J. Schachter and H. D. Caldwell, *Annu. Rev. Microbiol.* **34**, 285 (1980).
2. C. R. Dawson and J. Schachter, *Rev. Infect. Dis.*, in press.
3. J. Schachter, *N. Engl. J. Med.* **298**, 428 (1978).
4. H. Osake, *Acta Arachnol.* **25**, 23 (1973).
5. J. C. Harshbarger, S. C. Chang, S. V. Otto, *Science* **196**, 666 (1977).
6. G. I. Byrne and J. W. Moulder, *Infect. Immun.* **19**, 598 (1978).
7. R. R. Friis, *J. Bacteriol.* **110**, 706 (1972).
8. D. T. Kingsbury and E. Weiss, *ibid.* **96**, 1421 (1968).
9. H. D. Caldwell, C. C. Kuo, G. E. Kenny, *J. Immunol.* **115**, 963 (1975).
10. M. Nurminen, M. Leinonen, P. Saikku, P. H. Mäkelä, *Science* **220**, 1279 (1983).
11. M. Nurminen *et al.*, *Infect. Immun.* **44**, 609 (1984).
12. H. D. Caldwell and P. J. Hitchcock, *ibid.*, p. 306.
13. Sau 3A partially digested *C. trachomatis* DNA was sized by agarose gel electrophoresis, and DNA of 4 to 6 kilobases was pooled and cloned into Bam HI–digested, alkaline-phosphatased pUC8 [J. Vieira and J. Messing, *Gene* **19**, 259 (1982)]. The gene bank was screened essentially as described in D. M. Helfman *et al.* [*Proc. Natl. Acad. Sci. U.S.A.* **80**, 31 (1983)].
14. M. J. Osborn, in *Bacterial Outer Membranes*, M. Inouye, Ed. (Wiley, New York, 1979), p. 15.
15. P. H. Mäkelä and B. A. D. Stocker, *Symp. Soc. Gen. Microbiol.* **31**, 219 (1981).
16. E. S. Creeger and L. I. Rothfield, *J. Biol. Chem.* **254**, 804 (1979).
17. H. Watanabe, A. Nakamura, K. N. Timmis, *Infect. Immun.* **46**, 55 (1984).
18. S. K. Kadam, A. Rehemtulla, K. E. Sanderson, *J. Bacteriol.* **161**, 277 (1985).
19. J. T. Grayston and S. P. Wang, *J. Infect. Dis.* **132**, 87 (1975).
20. O. Westphal and K. Jann, *Methods Carbohydr. Chem.* **5**, 83 (1965).
21. G. Frens, *Nature (London) Phys. Sci.* **241**, 20 (1973).
22. E. N. Robinson *et al.*, *Infect. Immun.* **46**, 361 (1984).
23. We thank F. Hayes for performing the electron microscope work, S. Stewart and J. Simmons for technical help, S. Smaus for secretarial work, and the Laboratory of Microbial Structure and Function manuscript review committee for suggestions.

20 November 1984; accepted 14 February 1985