W1 IN406
NO.2 1986
-SEQ: 112940000
INFECTION AND IMMUNITY

1 • FEBRUARY 1986 • NUMBER 2

Infection and Immunity

NATIONAL LIBRARY
JAN 29 1986
OF MEDICINE

Published monthly by the
American Society for Microbiology

CODEN: INFIBR 51 (2) 373–721 (1986) ISSN: 0019-9567

INFECTION AND IMMUNITY

VOLUME 51 • FEBRUARY 1986 • NUMBER 2

J. W. Shands, Jr., *Editor in Chief* (1989)
University of Florida, Gainesville

Phillip J. Baker, *Editor* (1990)
National Institute of Allergy and Infectious Diseases
Bethesda, Md.

Edwin H. Beachey, *Editor* (1988)
VA Medical Center
Memphis, Tenn.

Peter F. Bonventre, *Editor* (1989)
University of Cincinnati
Cincinnati, Ohio

Roy Curtiss III, *Editor* (1990)
Washington University
St. Louis, Mo.

Arthur G. Johnson, *Editor* (1986)
University of Minnesota, Duluth

Stephan E. Mergenhagen, *Editor* (1989)
National Institute of Dental Research
Bethesda, Md.

EDITORIAL BOARD

Leonard C. Altman (1986)
Michael A. Apicella (1988)
Roland Arnold (1987)
John B. Bartlett (1988)
Joel B. Baseman (1988)
Robert E. Baughn (1987)
Gary K. Best (1988)
Jenefer Blackwell (1988)
Arnold S. Bleiweis (1987)
William H. Bowen (1988)
David E. Briles (1988)
Robert R. Brubaker (1986)
Gerald Byrne (1988)
Bruce Chassy (1987)
John O. Cisar (1988)
William B. Clark (1988)
John Clements (1988)
Don B. Clewell (1987)
Myron S. Cohen (1987)
R. John Collier (1987)
Jorge H. Crosa (1988)
Jim E. Cutler (1987)
James B. Dale (1988)
Judith E. Domer (1986)
James L. Duncan (1988)
Dennis Dwyer (1987)
Roman Dziarski (1987)
Robert A. Eisenberg (1987)
Barry Eisenstein (1988)
Toby K. Eisenstein (1987)
Peter Elsbach (1986)
Stanley Falkow (1988)
John R. Finerty (1987)
Robert Fitzgerald (1986)
James D. Folds (1988)
Samuel B. Formal (1986)
Peter Gemski (1988)
Robert Genco (1988)
Ronald J. Gibbons (1988)
Emil Gotschlich (1988)
Frank Griffin (1987)
Richard Guerrant (1986)
Thomas L. Hale (1987)
Robert E. W. Hancock (1988)
Edgar Hanna (1987)
Eric J. Hansen (1986)
Dexter H. Howard (1988)
Thomas P. Hatch (1988)
David Hentges (1988)
Randall K. Holmes (1986)
Marcus A. Horwitz (1987)
Vincent J. Iacono (1988)
Barbara Iglewski (1986)
Richard E. Isaacson (1987)
Howard M. Johnson (1988)
Russell C. Johnson (1988)
William Johnson (1988)
Dennis L. Kasper (1988)
F. Kierszenbaum (1987)
Paul Kolenbrander (1986)
Julius P. Kreier (1986)
Maurice J. Lefford (1987)
Thomas Lehner (1986)
Stephen H. Leppla (1988)
F. Y. Liew (1988)
Francis L. Macrina (1988)
John Mansfield (1988)
Jerry R. McGhee (1988)
Floyd C. McIntire (1988)
Monte Meltzer (1986)
Jiri Mestecky (1986)
J. Gabriel Michael (1986)
Henry W. Murray (1986)
Carol A. Nacy (1987)
Alison O'Brien (1988)
Itzhak Ofek (1986)
Andrew B. Onderdonk (1987)
Monique Parant (1987)
Shelley M. Payne (1988)
Nathaniel F. Pierce (1986)
Matthew Pollack (1986)
Reuben Ramphal (1988)
Richard R. Ranney (1988)
Ruth Rappaport (1986)
John B. Robbins (1986)
Donald Robertson (1987)
Burton Rosan (1986)
Catherine Saelinger (1987)
Dwayne C. Savage (1987)
Irving E. Salit (1986)
Charles F. Schachtele (1988)
Julius Schachter (1986)
Gary K. Schoolnik (1987)
June R. Scott (1987)
Philip Scott (1988)
Alan Sher (1987)
Gerald D. Shockman (1986)
W. A. Simpson (1988)
Phillip D. Smith (1988)
Ralph Snyderman (1988)
Maggie So (1986)
P. Frederick Sparling (1987)
Barnet M. Sultzer (1988)
Catharina Svanborg-Edén (1988)
John L. Swanson (1987)
Diane Taylor (1987)
K. N. Timmis (1987)
Ivo van de Rijn (1987)
Johannes van Houte (1988)
Stefanie Vogel (1988)
Peter A. Ward (1988)
William P. Weidanz (1987)
P. H. Williams (1987)
Jerry A. Winkelstein (1987)
Herbert H. Winkler (1986)
Elizabeth J. Ziegler (1988)
Wendell D. Zollinger (1988)

Helen R. Whiteley, *Chairman, Publications Board*

Linda M. Illig, *Managing Editor, Journals* **Thomas M. Rogers,** *Production Editor*

Infection and Immunity (ISSN 0019-9567), a publication of the American Society for Microbiology, 1913 I St., NW, Washington, DC 20006, is devoted to the advancement and dissemination of fundamental knowledge concerning: (i) infections caused by pathogenic bacteria, fungi, and unicellular parasites; (ii) the ecology and epidemiology of pathogenic microbes; (iii) virulence factors, such as toxins and microbial surface structures; (iv) nonspecific factors in host resistance and susceptibility to infection; and (v) immunology of microbial infection. Instructions to authors are published in the January issue each year; reprints are available from the editors and the Publications Department. *Infection and Immunity* is published monthly, and the twelve numbers are divided into four volumes per year. The nonmember subscription price is $280 per year; single copies are $21. The member subscription price is $41 (foreign, $54 [surface rate]) per year; single copies are $7. Correspondence relating to subscriptions, reprints, defective copies, availability of back issues, lost or late proofs, disposition of submitted manuscripts, and general editorial matters should be directed to the ASM Publications Department, 1913 I St., NW, Washington, DC 20006 (phone: 202 833-9680).

Claims for missing issues from residents of the United States, Canada, and Mexico must be submitted within 3 months after publication of the issues; residents of all other countries must submit claims within 6 months of publication of the issues. Claims for issues missing because of failure to report an address change or for issues "missing from files" will not be allowed.

Second class postage paid at Washington, DC 20006, and at additional mailing offices.

POSTMASTER: Send address changes to *Infection and Immunity*, ASM, 1913 I St., NW, Washington, DC 20006.

Made in the United States of America.

Copyright © 1986, American Society for Microbiology. 日本：価格は外貨表示とは関係なく円建。
All Rights Reserved.

The code at the top of the first page of an article in this journal indicates the copyright owner's consent that copies of the article may be made for personal use or for personal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per-copy fee through the Copyright Clearance Center, Inc., 21 Congress St., Salem, MA 01970, for copying beyond that permitted by Sections 107 and 108 of the U.S. Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale.

INFECTION AND IMMUNITY

Volume 51 February 1986 No. 2

BACTERIAL INFECTIONS AND IMMUNITY

Bactericidal Effect of Lactoferrin on *Legionella pneumophila*. Carol A. Bortner, Richard D. Miller, and Roland R. Arnold 373–377

Utilization of Anion-Exchange Chromatography and Monoclonal Antibodies to Characterize Multiple Pilus Types on a Uropathogenic *Escherichia coli* O6 Isolate. Rita M. Gander and Virginia L. Thomas 385–393

Involvement of Birds in the Epidemiology of the Lyme Disease Agent *Borrelia burgdorferi*. John F. Anderson, Russell C. Johnson, Louis A. Magnarelli, and Fred W. Hyde..... 394–396

Serogroup Specificity of *Legionella pneumophila* Is Related to Lipopolysaccharide Characteristics. Carol A. Ciesielski, Martin J. Blaser, and Wen-lan L. Wang 397–404

Morphological and Biochemical Comparison of Virulent and Avirulent Isolates of *Haemophilus pleuropneumoniae* Serotype 5. Allen E. Jensen and Timothy A. Bertram 419–424

Isolation, Purification, and Partial Characterization of a Lipopolysaccharide from *Haemophilus pleuropneumoniae*. James R. Maudsley, Solomon Kadis, and William R. Mayberry 501–506

Release of Fibronectin-Lipoteichoic Acid Complexes from Group A Streptococci with Penicillin. Timothy J. Nealon, Edwin H. Beachey, Harry S. Courtney, and W. Andrew Simpson 529–535

Human Antibody Response to a Group B Serotype 2a Meningococcal Vaccine Determined by Immunoblotting. Elisabeth Wedege and L. Oddvar Frøholm 571–578

Analysis of Damage to Human Ciliated Nasopharyngeal Epithelium by *Neisseria meningitidis*. David S. Stephens, Anne M. Whitney, M. Ann Melly, Loren H. Hoffman, Monica M. Farley, and Carl E. Frasch 579–585

Purification and Subunit Heterogeneity of Pili of *Bordetella bronchiseptica*. Simon W. Lee, Alice W. Way, and Eric G. Osen 586–593

Analysis of P Fimbriae on *Escherichia coli* O2, O4, and O6 Strains by Immunoprecipitation. Auli Pere, Vuokko Väisänen-Rhen, Mikael Rhen, Jukka Tenhunen, and Timo K. Korhonen 618–625

Superoxide Dismutase and Oxygen Toxicity Defenses in the Genus *Neisseria*. Frederick S. Archibald and Minh-Ngoc Duong..... 631–641

Experimental Meningococcal Infection in Mice: A Model for Mucosal Invasion. Irving E. Salit and Lewis Tomalty..... 648–652

Immunochemical and Biochemical Analysis of the Polyvalent *Pseudomonas aeruginosa* Vaccine PEV. Sheila MacIntyre, Treasa McVeigh, and Peter Owen 675–686

The Use of Mice in the Sereny Test as a Virulence Assay of Shigellae and Enteroinvasive *Escherichia coli*. Somay Yamagata Murayama, Takashi Sakai, Souichi Makino, Takeshi Kurata, Chihiro Sasakawa, and Masanosuke Yoshikawa..... 696–698

Continued on following page

Corteva Exhibit 2037 - Page 3 of 14
Bayer v. Corteva - IPR2023-01036

Continued from preceding page

Molecular Characterization of *Chlamydia trachomatis* and *Chlamydia psittaci* Plasmids. Theresa Joseph, Francis E. Nano, Claude F. Garon, and Harlan D. Caldwell 699–703

FUNGAL AND PARASITIC INFECTIONS AND IMMUNITY

Influence of Source and Quantity of Protein on the Development of Immunity and Resistance to African Trypanosomiasis. Jerry D. Norton, S. P. Yang, and Peter Diffley 455–460

Correlation of Natural Killer Cell Activity and Clearance of *Cryptococcus neoformans* from Mice after Adoptive Transfer of Splenic Nylon Wool-Nonadherent Cells. Michelle R. Hidore and Juneann W. Murphy 547–555

Antibody-Dependent Natural Killer Cell-Mediated Growth Inhibition of *Cryptococcus neoformans*. Nasrin Nabavi and Juneann W. Murphy..... 556–562

Conditioned Medium from Stimulated Mononuclear Leukocytes Augments Human Neutrophil-Mediated Killing of a Virulent *Acanthamoeba* sp. Antonio Ferrante and Tessa J. Abell..... 607–617

Description and Characterization of a Surface Lectin from *Giardia lamblia*. Michael J. G. Farthing, Miercio E. A. Pereira, and Gerald T. Keusch..... 661–667

Evidence for Macrophage-Mediated Protection against Lethal *Candida albicans* Infection. F. Bistoni, A. Vecchiarelli, E. Cenci, P. Puccetti, P. Marconi, and A. Cassone 668–674

Protection of Guinea Pigs against Cutaneous Leishmaniasis by Combined Infection and Chemotherapy. Joseph El-On, Avital Witztum, and Lionel F. Schnur 704–706

ORAL MICROBIOLOGY AND IMMUNITY

Purification of Lipoteichoic Acid by Chromatography in Water-Organic Solvent Systems. Stephen L. Josephson, Murray W. Stinson, Stephen J. Millar, and Robert E. Cohen 378–384

Isolation and Characterization of a 60-Kilodalton Salivary Glycoprotein with Agglutinating Activity against Strains of *Streptococcus mutans*. Jegdish P. Babu, Edwin H. Beachey, David L. Hasty, and W. Andrew Simpson..... 405–413

Immunological Cross-Reactivity of Anionic Proteins from Caries-Free and Caries-Active Salivas Which Differ in Biological Properties toward Oral Streptococci. S. D. Obenauf, R. A. Cowman, and R. J. Fitzgerald 440–444

Identification and Preliminary Characterization of a Lectinlike Protein from *Capnocytophaga gingivalis* (Emended). Angelika Kagermeier and Jack London 490–494

Human Immune Responses to Oral Microorganisms: Patterns of Systemic Antibody Levels to *Bacteroides* Species. J. L. Ebersole, M. A. Taubman, D. J. Smith, and Deirdre E. Frey..... 507–513

Human Serum Antibody Response against *Streptococcus mutans* Antigens. Michael J. Pucci, John G. Tew, and Francis L. Macrina 600–606

Adherence of Oral Treponemes and Their Effect on Morphological Damage and Detachment of Epithelial Cells In Vitro. Frans M. J. Reijntjens, Frans H. M. Mikx, Johanna M. L. Wolters-Lutgerhorst, and Jaap C. Maltha 642–647

Ability of Oral Bacteria to Degrade Fibronectin. Maude Wikström and Anders Linde 707–711

PATHOGENESIS

Relationship of Critical Micelle Concentrations of Bacterial Lipoteichoic Acids to Biological Activities. Harry S. Courtney, W. Andrew Simpson, and Edwin H. Beachey 414–418

Continued on following pag

Corteva Exhibit 2037 - Page 4 of 14
Bayer v. Corteva - IPR2023-01036

Continued from preceding page

Localization of Gonococcal Lipopolysaccharide and Its Relationship to Toxic Damage in Human Fallopian Tube Mucosa. Morris D. Cooper, Patricia A. McGraw, and M. Ann Melly 425–430

Affinity Purification of Staphylococcal Toxic Shock Syndrome Toxin 1 and Its Pathologic Effects in Rabbits. Michael W. Reeves, Robert J. Arko, Francis W. Chandler, and Nancy B. Bridges 431–439

Virulence Genes Regulated at the Transcriptional Level by Ca^{2+} in *Yersinia pestis* Include Structural Genes for Outer Membrane Proteins. Susan C. Straley and William S. Bowmer 445–454

Multiplication of *Shigella flexneri* within HeLa Cells: Lysis of the Phagocytic Vacuole and Plasmid-Mediated Contact Hemolysis. Philippe J. Sansonetti, Antoinette Ryter, Philippe Clerc, Anthony T. Maurelli, and Joelle Mounier 461–469

Molecular Alteration of the 140-Megadalton Plasmid Associated with Loss of Virulence and Congo Red Binding Activity in *Shigella flexneri*. Chihiro Sasakawa, Kunio Kamata, Takashi Sakai, Somay Y. Murayama, Souichi Makino, and Masanosuke Yoshikawa 470–475

Molecular Cloning of a Genetic Determinant for Congo Red Binding Ability Which is Essential for the Virulence of *Shigella flexneri*. Takashi Sakai, Chihiro Sasakawa, Souichi Makino, Kunio Kamata, and Masanosuke Yoshikawa 476–482

***Mycobacterium leprae* Fails to Stimulate Phagocytic Cell Superoxide Anion Generation.** Timothy J. Holzer, Kenrad E. Nelson, Victoria Schauf, Ray G. Crispen, and Burton R. Andersen 514–520

Localized Gut-Associated Lymphoid Tissue Hemorrhage Induced by Intravenous Peptidoglycan-Polysaccharide Polymers. R. Balfour Sartor, Sonia K. Anderle, William J. Cromartie, and John H. Schwab 521–528

Mucus Colonization as a Determinant of Pathogenicity in Intestinal Infection by *Campylobacter jejuni*: A Mouse Cecal Model. Adrian Lee, Jani L. O'Rourke, Patricia J. Barrington, and Trevor J. Trust 536–546

Interaction between Heat-Stable Hemolytic Substance from *Haemophilus pleuropneumoniae* and Porcine Pulmonary Macrophages In Vitro. Katsumi Kume, Toyotsugu Nakai, and Akira Sawata 563–570

Environmental Modulation of Lipopolysaccharide Chain Length Alters the Sensitivity of *Escherichia coli* to the Neutrophil Bactericidal/Permeability-Increasing Protein. Jerrold Weiss, Michael Hutzler, and Linda Kao 594–599

Plasmids Coding for Colonization Factor Antigens I and II, Heat-Labile Enterotoxin, and Heat-Stable Enterotoxin in *Escherichia coli*. Peter Echeverria, Jitvimol Seriwatana, David N. Taylor, Suchitra Changchawalit, Cyril J. Smyth, Jane Twohig, and Bernard Rowe 626–630

Antigenic Heterogeneity in High- and Low-Virulence Strains of *Rickettsia rickettsii* Revealed by Monoclonal Antibodies. Robert L. Anacker, Robert H. List, Raymond E. Mann, and Danny L. Wiedbrauk 653–660

In Vivo Identification of Sialic Acid as the Ocular Receptor for *Pseudomonas aeruginosa*. L. D. Hazlett, M. Moon, and R. S. Berk 687–689

Antigenic Determinants of the Attachment Protein of *Mycoplasma pneumoniae* Shared by Other Pathogenic *Mycoplasma* Species. Wallace A. Clyde, Jr. and Ping C. Hu 690–692

Chromosomal Map Position of Genes Encoding P Adhesins in Uropathogenic *Escherichia coli*. R. Hull, S. Bieler, S. Falkow, and S. Hull 693–695

Continued on following page

Continued from preceding page

Plasmids Coding for Drug Resistance and Localized Adherence to HeLa Cells in Enteropathogenic *Escherichia coli* O55:H⁻ and O55:H6. Marcia Z. Laporta, M. Lourdes M. Silva, Isabel C. A. Scaletsky, and Luiz R. Trabulsi 715–717

IMMUNE MECHANISMS

Enhanced Recovery of Murine Alveolar Macrophages: Morphological and Functional Characteristics following Intravenous Injection of Heat-Killed *Mycobacterium bovis* BCG. Innocent N. Mbawuike, Jordan E. Luhr, and Herbert B. Herscowitz 483–489

Strain Variations in the Murine Cellular Immune Response to the Phenolic Glycolipid I Antigen of *Mycobacterium leprae*. Frederick T. Koster, Cory Teuscher, Patrick Matzner, Edith Umland, Donna Yanagihara, Patrick J. Brennan, and Kenneth S. K. Tung 495–500

Effects of Dexamethasone on Human Natural Killer Cell Cytotoxicity, Interferon Production, and Interleukin-2 Receptor Expression Induced by Microbial Antigens. Enza Piccolella, Giovanna Lombardi, Daniela Vismara, Flavia Del Gallo, Vittorio Colizzi, Antonina Dolei, and Ferdinando Dianzani 712–714

LETTER TO THE EDITOR

Results of a World Health Organization-Sponsored Workshop to Characterize Antigens Recognized by Mycobacterium-Specific Monoclonal Antibodies 718–720

AUTHOR'S CORRECTION

In Vitro Parasite-Monocyte Interactions in Human Leishmaniasis: Possible Role of Fibronectin in Parasite Attachment. David J. Wyler, Joseph P. Sypek, and John A. McDonald 721

Date of Issue: 24 January 1986

This material may be protected by Copyright law (Title 17 U.S. Code)

INFECTION AND IMMUNITY, Feb. 1986, p. 571–578
0019-9567/86/020571-08$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 51, No. 2

Human Antibody Response to a Group B Serotype 2a Meningococcal Vaccine Determined by Immunoblotting

ELISABETH WEDEGE* AND L. ODDVAR FRØHOLM

Department of Methodology, National Institute of Public Health, Geitmyrsveien 75, 0462 Oslo 4, Norway

Received 26 August 1985/Accepted 23 October 1985

The antibody response of 30 volunteers vaccinated with a complex of group B polysaccharide and outer membrane vesicles (OMV) from serotype 2a *Neisseria meningitidis* and of 3 individuals who received a placebo vaccine was determined by immunoblotting. OMV were separated by sodium dodecyl sulfate-gel electrophoresis and electrotransferred to nitrocellulose filters. Binding of immunoglobulin G (IgG), IgA, and IgM antibodies in the human sera to OMV components was detected with class-specific peroxidase-conjugated antibodies. The immunoblotting results were also related to the bactericidal activity of the sera and the meningococcal carrier status of the volunteers. Before vaccination weakly reactive bands in the molecular weight range of 140,000 to 10,000 were observed on the blots. Sera from carriers showed more marked bands. Individual patterns of increased reactivity were seen 6 weeks after vaccination. The main immunoreactive components of OMV corresponded to a molecular weight of 43,000 (class 1 protein), 30,000 (class 5 proteins), and 22,000. IgG antibodies in postvaccination sera of high bactericidal titers showed distinct binding to the 43,000-molecular-weight antigen. Meningococcal carriers had antibodies against an antigen of 22,000 molecular weight; in polyacrylamide gels this component did not stain with Coomassie brilliant blue or silver. The marked binding of IgG antibodies to the class 5 proteins decreased considerably between weeks 6 and 25 after vaccination. Periodate oxidation of OMV abolished the binding of IgG antibodies to the class 5 proteins, whereas the antigenicity of the 43,000-molecular-weight (class 1 protein) and 22,000-molecular-weight antigens was unaffected.

A meningococcal vaccine trial was carried out in Norway in 1981 and 1982. Thirty-three adults were given a vaccine consisting of noncovalent complexes of group B polysaccharide and outer membrane material from serotype 2a meningococci, whereas 27 volunteers received the placebo vaccine (6). The immune response of the individuals was evaluated by determination of the antibody levels (18) and the bactericidal activity of the sera (6; L. O. Frøholm, unpublished data). In the present communication, the antibody response was studied by an immunoblotting method. With this technique we wanted to investigate the immunogenicity of the different components in the outer membrane fraction in the vaccine and, if possible, to correlate the results with previous determinations of the immune response such as antibody levels and bactericidal activities of the sera (6, 18; Frøholm, unpublished data). It was also of interest to see if the meningococcal carrier status of the volunteers would influence the pattern of reactive bands on the immunoblots.

(The preliminary findings with sera from 15 of the vaccinees have been reported previously (E. Wedege, L. O. Frøholm, T. E. Michaelsen, and C. E. Frasch, The Pathogenic Neisseriae. Proceedings of the Fourth International Symposium, 1984, in press.)

MATERIALS AND METHODS

Vaccination sera. The volunteers received a vaccine dose of 0.5 ml consisting of: outer membrane vesicles (OMV) from *Neisseria meningitidis* M986 NCV-1 serotype 2a, 50 μg of protein and 8 μg of lipopolysaccharide (LPS); *N. meningitidis* group B polysaccharide, 50 μg; sodium thimerosal, 50 μg; lactose, 3 mg; and NaCl, 4.25 mg (lot 790626VB, obtained from C. E. Frasch, Center for Drugs and Biologics, Bethesda, Md.) (6). An identical dose was given 4 weeks after the first immunization. The placebo controls received sodium thimerosal, lactose, and NaCl only. The sera used for immunoblotting were those collected from the individuals at the day of the first vaccination (prevaccination sera) and after 6 and 25 weeks. They were stored at −25°C. Sera from 33 volunteers, 30 of the 33 vaccinees and 3 of the 27 placebo controls, were selected for the present investigation. Study of pharyngeal swabs of bacterial isolates showed that 55% of these individuals were meningococcal carriers at the time of vaccination (6). The bactericidal titers used in this report were determined with human complement (Frøholm, unpublished data). The numbers designating the volunteers are the same as published previously (6).

SDS-gel electrophoresis. The antigen in the immunoblotting experiments was an OMV fraction isolated from the same meningococcal strain as the vaccine OMV antigen (5). Its protein content was measured by the Folin method (11) with bovine serum albumin as the standard. OMV were boiled for 5 min in sample buffer with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol and separated in a discontinuous SDS-gel electrophoretic system (10). The samples contained pyronine B as a marker for the subsequent transblot electrophoresis. The separating and stacking gels contained 12 and 4% acrylamide, respectively. The dimensions of the 1.5-mm-thick gels were 14 by 12 cm. Electrophoresis through the separating gel was performed at 30 mA for 3 h in cold buffer. The proteins used as molecular weight standards were: phosphorylase *a* (94,000), bovine serum albumin (67,000), ovalbumin (45,000), lactate dehydrogenase (36,000), car-

* Corresponding author.

Corteva Exhibit 2037 - Page 7 of 14
Bayer v. Corteva - IPR2023-01036

bonic anhydrase (30,000), chymotrypsinogen A (25,000), RNase (14,000), and cytochrome *c* (12,000). SDS-gels were stained with Coomassie brilliant blue (13).

Detection of antibodies by immunoblotting. After SDS-gel electrophoresis, the separated OMV were transferred to nitrocellulose (NC) filters (0.45-μm pore size; Bio-Rad Laboratories, Richmond, Calif.) at 300 to 400 mA for 3 h, using cold Tris glycine buffer with 20% methanol (22). The blots were then cut into 0.5-cm-wide strips, each corresponding to approximately 5 μg of OMV protein before gel electrophoresis. Guide strips were stained with amido black (7). The other strips were soaked in a blocking buffer of 3% bovine serum albumin in physiological saline for 30 min before incubation overnight in pre- or postvaccination sera diluted 1:200 with the blocking buffer. Afterwards the blots were treated for 2 h with peroxidase-conjugated rabbit anti-human immunoglobulin G (IgG) (γ chains), IgA (α chains), or IgM (μ chains) (DAKOPATTS a/s, Denmark), diluted 1:500 with the blocking buffer. The strips were washed four times in physiological saline after each step. Binding of the enzyme-labeled antibodies was detected with 3-amino-9-ethylcarbazole and hydrogen peroxide (14). The blots with anti-IgG, anti-IgA, and anti-IgM antibodies were stained for 4, 10, and 20 min, respectively, and then rinsed in water and dried. The incubations and washings were performed at room temperature on a rotary shaker. No binding of the peroxidase-conjugated antisera to OMV was observed in control experiments.

A mouse monoclonal antibody, 3-1-P1.2 (provided by W. D. Zollinger) that reacted with class 1 protein from serotype 2 *N. meningitidis* strains (27) was used to identify this protein on NC strips. OMV were separated and electroblotted as described above and incubated overnight with the monoclonal antibody (1:1,000), followed by a 2-h incubation with peroxidase-conjugated sheep anti-mouse immunoglobulin (1:10,000) (provided by T. E. Michaelsen). The monoclonal antibody was omitted in the control during the overnight incubation. The antibodies in these studies were diluted with the same blocking buffer as described above.

Periodate oxidation of OMV. Periodate oxidation was carried out in the dark for 5 h at 33°C in 0.05 M sodium acetate (pH 5.0) as described by Spiro (19). The final concentration of sodium periodate was 0.07 or 0.035 M. The oxidation was stopped by adding an excess of ethylene glycol. The samples were dialyzed overnight in physiological saline before SDS-gel electrophoresis. In one control, OMV were added after the reduction of the periodate by ethylene glycol; in another, OMV were incubated for 5 h at 33°C in sodium acetate buffer without periodate to examine the stability of the antigen.

Statistical analysis. Student's *t* test was used to evaluate the significance of the differences in the serum antibody levels which had previously been measured by the enzyme-linked immunosorbent assay (ELISA) (18). Differences were considered to be significant when *P* values less than 0.05 were found.

RESULTS

Analysis of OMV. SDS-gels of the OMV preparation used in the vaccine and as immunoblotting antigen showed two main and two minor stained bands (Fig. 1, lane b). These bands corresponded from top to bottom to the class 1, 2, 4, and 5 proteins of the major outer membrane proteins (25) and were found to have molecular weights of 43,000, 41,000, 34,000, and 30,000, respectively. A similar pattern of bands

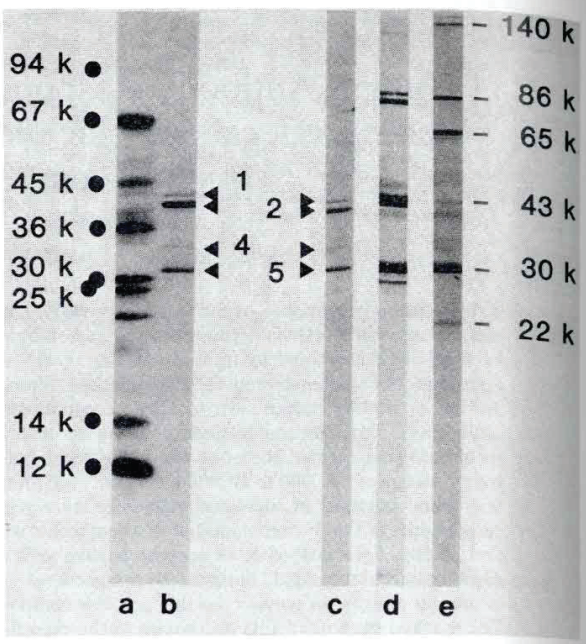


FIG. 1. SDS-gel electrophoretic and immunoblot analyses of OMV from *N. meningitidis*. SDS-gel of standard proteins (lane a) and OMV (lane b) stained with Coomassie brilliant blue. The dots mark the positions of the standard proteins used (from top to bottom: phosporylase *a*, bovine serum albumin, ovalbumin, lactate dehydrogenase, carbonic anhydrase, chymotrypsinogen A, RNase, and cytochrome *c*). Lane c: NC strip with electroblotted OMV stained with amido black. The protein classes 1, 2, 4, and 5 of OMV are marked with arrows. Lanes d and e: Immunoblots showing the different patterns of IgG antibody binding to the OMV with two individual sera (1:200) drawn 6 weeks after vaccination. The molecular weights of the main antigenic components are indicated to the right (k, 10^3). The immunoreactive bands were visualized with peroxidase-conjugated anti-human IgG (1:500).

was seen after electrotransfer to NC filters (Fig. 1, lane c). Blots incubated with 6-week postvaccination sera showed a more complex pattern of antigenic OMV components, and the individual sera seemed to recognize different antigens (Fig. 1, lanes d and e). IgG antibodies bound most markedly to OMV components that corresponded to molecular weights of 140,000, 86,000, 65,000, 43,000 (class 1 protein), 30,000 (class 5 proteins), and 22,000. The antigens of 140,000, 86,000, and 65,000 molecular weight, but not the 22,000-molecular weight antigen, were also observed as very faintly stained bands on SDS-gels.

IgG antibody response after vaccination. Figure 2 demonstrates the binding of IgG antibodies in sera collected 6 weeks after vaccination of the 33 volunteers in this study. The patterns of reactive bands varied considerably from individual to individual. The majority of the sera showed the strongest binding to the 30,000-molecular-weight antigen, but other OMV components of higher and lower molecular weight as described above were also recognized. Weakly stained bands were observed with sera from volunteers 1, 31, and 45, who only received one meningococcal vaccine dose, and with sera from the placebo controls 34 and 82.

IgG antibodies in the sera from volunteers 2, 42, 71, 87, 111, and 112 bound distinctly to the 43,000-molecular-weight antigen (Fig. 2). The bactericidal titers of these sera were

Corteva Exhibit 2037 - Page 8 of 14
Bayer v. Corteva - IPR2023-01036

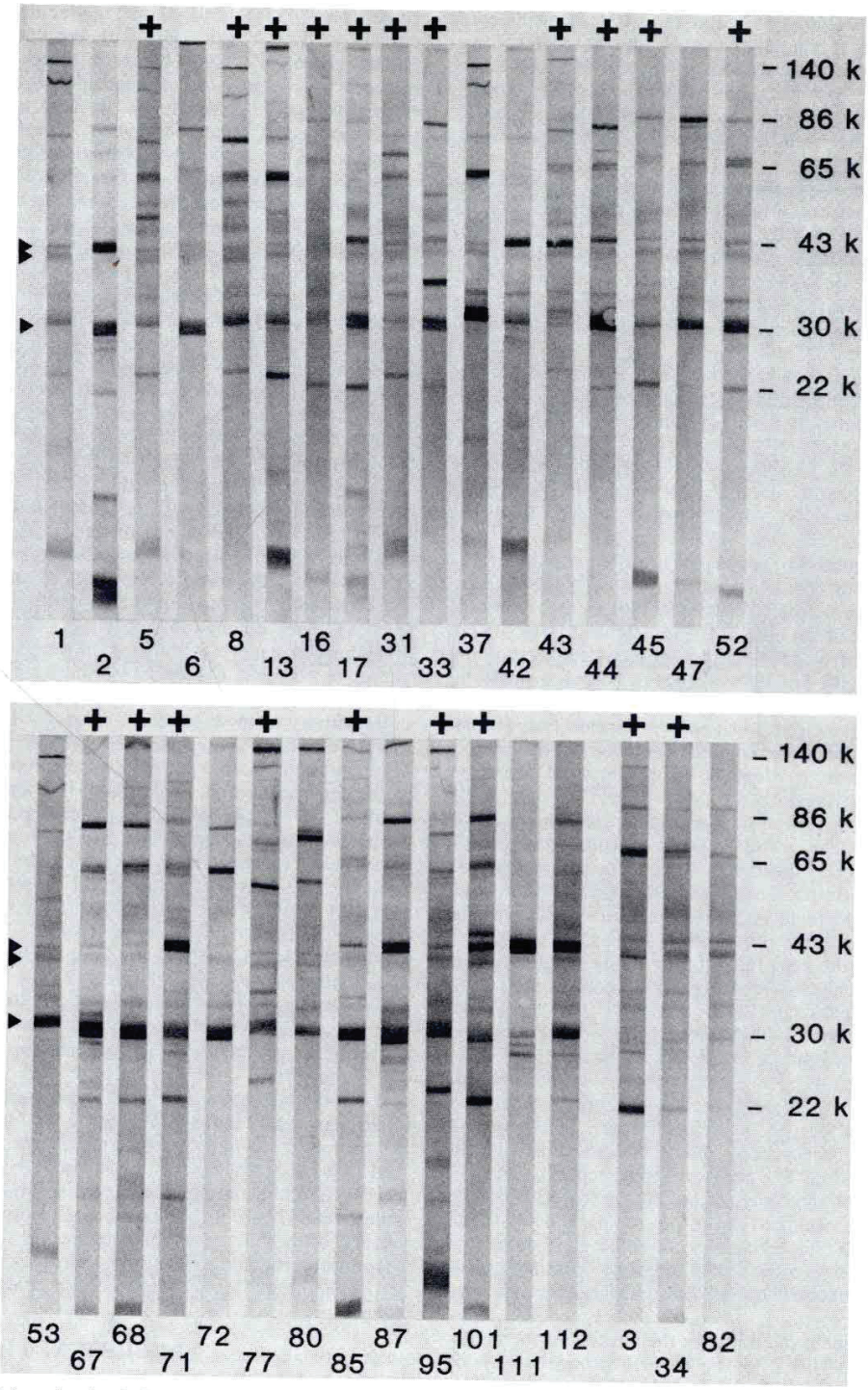


FIG. 2. Immunoblots showing IgG antibody activity against OMV from *N. meningitidis* in postvaccination sera from 30 vaccinees and 3 placebo controls. OMV were separated by SDS-gel electrophoresis, electroblotted to NC filters, and incubated with sera (1:200) drawn 6 weeks after the first vaccination. IgG antibody binding was visualized with peroxidase-conjugated anti-human IgG (1:500). The volunteer number designation is marked at the bottom of each strip; the + on the top of the strip indicates that the volunteer was a meningococcal carrier during the first 6 weeks after vaccination. The arrows to the left show (from top to bottom) the protein classes 1, 2, and 5, respectively. The molecular weights of the main antigenic components are seen to the right (k, 10^3). The strips represent several experiments, leading to some variations in the mobilities of the main bands. Volunteers 3, 34, and 82 were placebo controls, whereas 1, 31, and 45 only received one dose of meningococcal vaccine.

Corteva Exhibit 2037 - Page 9 of 14
Bayer v. Corteva - IPR2023-01036

1:64, 1:128, 1:64, 1:256, 1:512, and 1:>1,024, respectively. Only two sera of similar high activity did not detect this antigen (numbers 52 and 67 with titers of 1:512 and 1:64, respectively). The IgG antibodies from vaccinees 17, 43, and 101 showed moderate binding to the 43,000-molecular-weight antigen. The bactericidal titers of these sera ranged from 1:8 to 1:32.

Many of the 6-week postvaccination sera had IgG antibodies directed against an antigen of 22,000 molecular weight (Fig. 2). The antigen was not stained in SDS-gels with Coomassie brilliant blue or silver (24) or on NC filters with amido black. The presence of this band on immunoblots seemed to be correlated with meningococcal carriage of the volunteers as it was generally weaker or not detected with sera from noncarriers.

In Fig. 2 it can also be seen that several sera recognized low-molecular-weight components in the range about 10,000 to 8,000. These antigens probably represented lipopolysaccharides (LPS) in the OMV fraction (23). This assumption was supported by the detection of bands of similar molecular weight in SDS-gels after staining with alcian Blue or Stains-All (Eastman Kodak Co., Rochester, N.Y.) (E. Wedege, unpublished data).

IgA and IgM antibody responses after vaccination. The binding to OMV of IgA and IgM antibodies in the 6-week postvaccination sera from all volunteers was also studied. The results for six of the individuals are shown in Fig. 3, and they corresponded to previous findings (Wedege et al., in press). IgA antibodies in 40% of all sera bound strongly to one or more of the 140,000-, 86,000-, 43,000-, 30,000-, and 22,000-molecular-weight antigens, whereas the others showed weak bands. Marked IgA-reactive bands on the blots did not seem to depend on bactericidal titers or meningococcal carriage.

IgM antibodies in 30% of the sera recognized one or more of the 43,000-, 30,000-, and 22,000-molecular-weight antigens. The majority of these sera were from meningococcal carriers. No relationship with the bactericidal titers was observed. The IgM-reactive bands were usually less strong than those seen with the other antibody classes. Compared with the IgA antibodies, the IgM antibodies bound more often to LPS antigens but less often to antigens of molecular weights above 43,000.

Periodate oxidation of OMV. When OMV were oxidized with periodate, the binding of IgG antibodies to the 43,000- and 22,000-molecular-weight antigens was unaffected, whereas that to the 30,000-molecular-weight antigen was reduced or abolished (Fig. 4, lanes c to f). Often several weak bands were observed in the 30,000-molecular-weight region after oxidation. The periodate treatment also affected the antigenicity of the components of 86,000, 65,000, and 34,000 molecular weight (class 4 protein) and the LPS antigens. The two concentrations of sodium periodate used were equally effective, and the antibody binding to the controls was similar to that of the untreated sample (see above).

Periodate treatment did not alter the binding of the monoclonal antibody 3-1-P1.2 to the class 1 protein of 43,000 molecular weight (Fig. 4, lanes g and h). However, both this band and that obtained after incubation with a serum of high bactericidal activity were somewhat more diffuse after oxidation (compare lanes e and f and lanes g and h in Fig. 4).

SDS-gels stained with Coomassie brilliant blue showed only faint traces of the 30,000-molecular-weight protein after periodate treatment of OMV (Fig. 4, lanes a and b). The 34,000-molecular-weight protein was fainter, whereas the proteins of 43,000 and 41,000 molecular weight stained equally well after oxidation. The staining intensity of the 30,000-molecular-weight band in the two controls (see above) was similar to that of untreated OMV, indicating that no visible proteolysis of this protein took place during incubation and dialysis.

Comparison of antibody distribution in pre- and postvaccination sera. The effect of vaccination was studied by comparing the binding patterns of each antibody class before and 6 weeks after vaccination of the 33 volunteers. The results for volunteers 13, 85, and 87 have been reported previously (Wedege, et al., in press). The blots for six other volunteers, two of whom received the placebo vaccine (numbers 3 and 34), are presented in Fig. 3. The figure also shows the IgG antibody activity of sera drawn 25 weeks after the first vaccination. Generally, the prevaccination sera showed faint bands on the blots that corresponded to the major outer membrane proteins of molecular weights of 43,000, 41,000, 34,000, and 30,000, but other weak bands in the molecular weight range of 140,000 to 10,000 were also observed. Several prevaccination sera demonstrated more distinct binding of IgG, IgA, or IgM antibodies (see numbers 68, 71, and 80 in Fig. 3 as examples); 69, 67, and 82% of such sera, respectively, were from meningococcal carriers. No relationship to the bactericidal titers of the sera was noted. Increased antibody binding to many of the antigens was seen 6 weeks after vaccination (Fig. 3). As described above, the 30,000-molecular-weight antigen seemed to be most antigenic, in addition to the 43,000-molecular-weight antigen recognized by IgG antibodies in the serum of high bactericidal activity from volunteer 71.

Figure 3 also shows that the prevaccination sera from volunteers 34, 68, and 71, who were meningococcal carriers at the time of the first vaccination, had antibodies of one or more of the immunoglobulin carrier classes to the 22,000-molecular-weight antigen. The possible relationship between carriage and this band is further demonstrated by referring to individuals 3 and 80 in the figure. Volunteer 3 became a carrier 4 weeks after the first vaccination, and volunteer 80 became a carrier sometime between weeks 6 and 25. The blots showed IgG antibodies in their corresponding postvaccination sera that bound distinctly to the 22,000-molecular-weight antigen. After the placebo vaccination of volunteers 3 and 34, the staining intensities of the other bands did not increase, in contrast to that observed for the vaccinees 53, 68, 71, and 80.

Time-related decrease in antibody binding to the 30,000-molecular-weight antigen. When the binding of IgG antibodies to the 30,000-molecular-weight antigen was compared 6 and 25 weeks after vaccination, a marked decrease with time was seen in the staining intensity of this band with sera from volunteers 53, 68, 71, and 80 (Fig. 3). Similar findings were observed with all sera that showed strong IgG antibody response to the antigen 6 weeks after vaccination. The other antigens did not demonstrate a corresponding decrease with time.

DISCUSSION

Immunoblotting has found wide applications in the study of bacterial and viral infections (as reviewed by Towbin and Gordon [21]). We used the method to investigate the immune response in human volunteers who received a group B serotype 2a meningococcal vaccine (6). OMV in the vaccine were resolved by SDS-gel electrophoresis and electrotransferred to NC filters for immunoperoxidase staining with sera from 33 volunteers. The use of secondary

Corteva Exhibit 2037 - Page 10 of 14
Bayer v. Corteva - IPR2023-01036

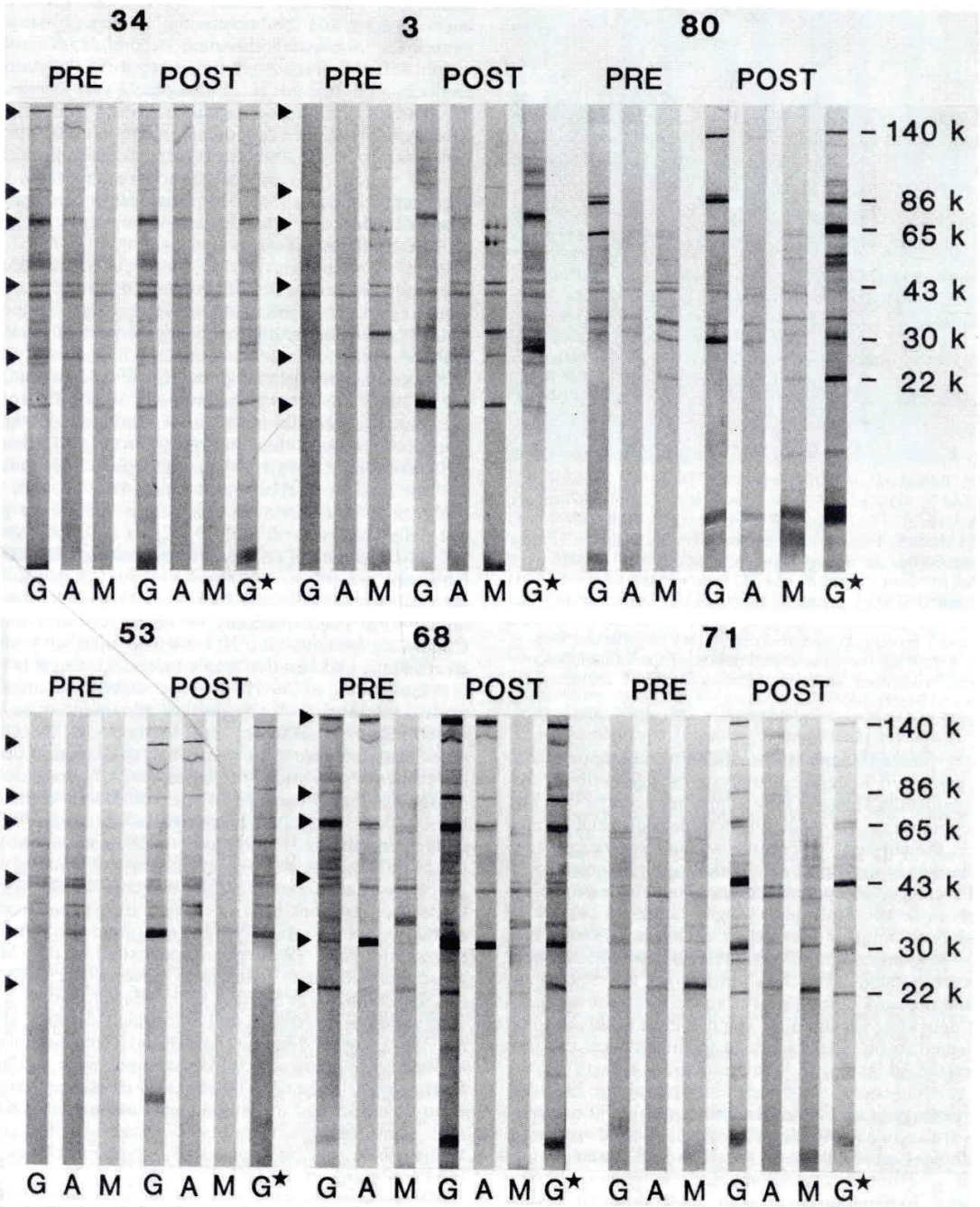


FIG. 3. Antibody activity of pre- and postvaccination sera against OMV components from *N. meningitidis*. The binding to OMV of IgG, IgA, and IgM antibodies in pre (PRE)- and post (POST)-vaccination sera (1:200) is marked by G, A, and M, respectively, at the bottom of the strips. The postvaccination sera were collected 6 weeks after the first vaccination. G* represents IgG antibody binding of the 25-week postvaccination sera. The volunteer number designation is shown on top of the blots. The molecular weights of the main antigenic components of OMV are indicated to the right (k, 10^3), and the arrows show the corresponding bands for each volunteer. The immunoblots were from several experiments, causing some variation in the mobilities of the main antigens. Strips showing IgG antibody binding of the 6- and 25-week postvaccination sera from each volunteer were from the same experiment. Volunteers 3 and 34 received the placebo vaccine, whereas 53, 68, 71, and 80 were vaccinated twice. Volunteer 53 did not carry meningococci (see Results).

Corteva Exhibit 2037 - Page 11 of 14
Bayer v. Corteva - IPR2023-01036

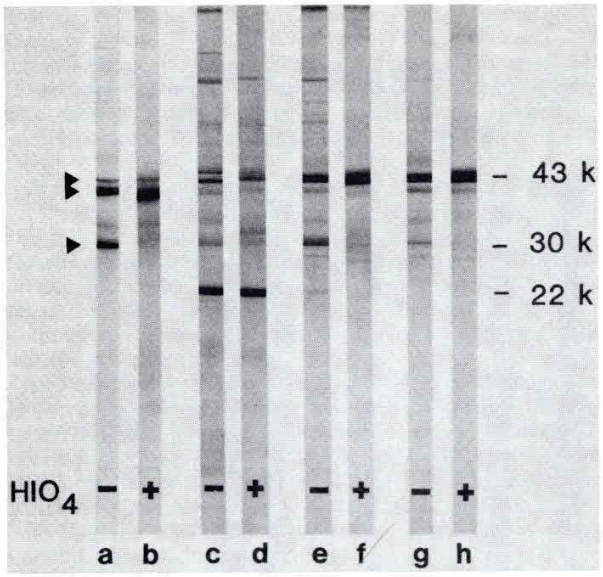


FIG. 4. Periodate oxidation of OMV from *N. meningitidis*. Lanes a and b: SDS-gels of OMV stained with Coomassie brilliant blue. The class 1, 2, and 5 proteins are indicated with arrows (top to bottom) to the left. Lanes c to f: Immunoblots showing the binding of IgG antibodies in 6-week postvaccination sera (1:200) from volunteers 101 (lanes c and d) and 112 (bactericidal titer 1:>1,024) (lanes e and f). Lanes g and h: Immunoblots demonstrating the binding of the monoclonal antibody 3-1-P1.2 (1:1,000) directed against class 1 protein. Periodate-treated OMV are marked with +. All strips were from the same experiment, and the immunoreactive bands were visualized with peroxidase-conjugated antisera (see Materials and Methods).

antibodies directed against the different immunoglobulin classes permitted a detailed analysis of the antibody response against the various OMV components in the vaccine.

The immunoblots showed considerable individual heterogeneity in the antibody response 6 weeks after vaccination. IgG antibodies in some postvaccination sera recognized only one OMV component, whereas other sera detected up to six components in the molecular weight range of 140,000 to 10,000. Fewer antigens seemed to elicit the IgA and IgM antibody response. The 30,000-molecular-weight antigen, which corresponded to the class 5 proteins of the major outer membrane proteins (25), appeared to be the most immunogenic protein after vaccination. The presence of this band on blots seemed to be independent of meningococcal carriage and bactericidal activity. Weak antibody activity to the protein was observed in the individuals receiving only one vaccination dose and in the placebo controls. The class 5 proteins are known to induce a strong IgG antibody response after meningococcal infection (16). However, Zollinger and Mandrell (26) obtained evidence for the response being lower after vaccination than after infection. Our results suggested that the antibody levels against these proteins decreased with time after vaccination. This and the fact that the class 5 proteins are variable (15) probably indicate that they are of less importance as immunogens in a vaccine.

One of our main findings was the strong IgG antibody binding to the 43,000-molecular-weight antigen with sera of high bactericidal activity. This was observed with six of the eight sera of titers >1:64, and five of these also had IgA antibodies directed against the protein. The antigen corresponded to the class 1 protein, which was confirmed with the monoclonal antibody 3-1-P1.2 (Fig. 4). It is therefore tempting to suggest that the bactericidal activity of many sera is caused by antibodies directed to the 43,000-molecular-weight antigen. This assumption is supported by studies with monoclonal antibodies (L. F. Mocca, A. B. Karpas, L. H. Wu, and C. E. Frasch, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 295, 1984) and the observation of antibody binding to the class 1-class 2 protein region on blots with one human serum of high bactericidal activity (26). The relationship between class 1 protein antibodies and bactericidal activity may be important in the formulation of new vaccines.

Periodate treatment of OMV did not affect the binding of IgG antibodies to the class 1 protein. Therefore, the majority of the epitopes on this antigen reacting with antibodies in sera of high bactericidal activity were probably not carbohydrates or amino acids susceptible to oxidation (2, 19). The same conclusion might be drawn for the single epitope that reacted with the monoclonal antibody 3-1-P1.2. In contrast, the class 5 protein did not bind IgG antibodies or stain with Coomassie brilliant blue after oxidation (Fig. 4). Cleavage of the protein might explain these findings, but peptide bonds are not likely to be broken by periodate (2, 19). LPS is reported to associate with some outer membrane proteins even after boiling with SDS (9, 17). If LPS interacted with the class 5 proteins, and the corresponding band of 30,000-molecular weight on the blots was due to IgG antibodies directed primarily against LPS, then the antigenicity of the bound LPS would probably be destroyed after oxidation. Coomassie brilliant blue is known to bind to proteins by electrostatic and van der Waals forces (3), and it is possible that the binding of the dye was prevented by changes in the protein molecule after periodate treatment. The control experiments (see Materials and Methods and Results) indicated that the class 5 protein was not degraded under the experimental conditions in the absence of periodate.

Meningococcal carriage of the volunteers seemed to be associated with antibody activity to an antigen of 22,000 molecular weight. The reactivity of the component was not destroyed by periodate. The antigen had unusual staining properties because it was not detected in SDS-gels with Coomassie brilliant blue or silver. Its gel electrophoretic mobility was not modified by treatment with heat or 2-mercaptoethanol (Wedege, unpublished data). Many of these characteristics resembled those of the CA2 antigen (W. D. Zollinger, J. S. Ray, E. E. Moran, and R. Seid, 4th International Conference on Pathogenic Neisseria, abstr. no. 58, 1984), the H.8-binding antigen (1), and an antigen described by Sugasawara (20) in meningococcal strains. Antibodies against pili of 16,000 to 21,000 molecular weight have been demonstrated in patients with meningococcal disease (16). These antigens were also not stained with Coomassie brilliant blue, but, in contrast to our findings, they did not bind antibodies of the IgG class (16).

Immunoblotting is not a strictly quantitative method, but it gives some indications of the relative antibody levels present and the antibody avidity (8). Each NC strip in our experiments corresponded to 5 μg of OMV protein applied before gel electrophoresis. Distinct protein bands were seen in gels stained with Coomassie brilliant blue after electrotransfer, implying that the blotting conditions left a considerable part of the OMV in the gels. This indicated that much less than 1 μg of the 140,000-, 86,000-, 65,000-, and 43,000-molecular-weight protein components was strongly antigenic with a

Corteva Exhibit 2037 - Page 12 of 14
Bayer v. Corteva - IPR2023-01036

1:200 dilution of several postvaccination sera. The class 2 protein of 41,000 molecular weight, which is the serotype protein of the meningococcal strain M986 (4), showed only weak antibody binding after vaccination. The antigenicity of this protein is readily lost upon denaturation to the monomeric form (16, 27) but may be partly restored upon inclusion of dipolar ionic detergent during the blotting procedure (12). Therefore, the antibody response detected by the present immunoblotting method will probably underestimate the immunological importance of the class 2 protein and possibly other antigens in the vaccine.

Rosenqvist et al. (18) have previously measured the levels of the different immunoglobulin classes in the individual sera by an ELISA. The antigen was an OMV fraction from the same meningococcal strain as used for immunoblotting. It was of interest to see whether there was a correspondence between high antibody levels determined by ELISA and marked immunoreactive bands on blots for the individual sera. We found that prevaccination sera, which showed distinct binding of IgG, IgA, or IgM antibodies to blots, had significantly higher levels of the respective antibodies than those giving weaker bands. As described above, the majority of these sera were from meningococcal carriers. This was in reasonable agreement with the ELISA observations showing that the levels before vaccination of IgG and IgA, but not IgM antibodies, were higher in meningococcal carriers than noncarriers (18).

The amount of IgA antibodies in the 6-week postvaccination sera which demonstrated distinct IgA-reactive bands on blots was also significantly higher than those yielding weak bands. Such a difference was not observed for the IgM antibodies binding to various OMV components or for the IgG antibodies that bound strongly to the 43,000-, 30,000-, or 22,000-molecular-weight antigens. The majority of the postvaccination sera that showed IgM antibody activity on blots were from carriers, whereas such correspondence was not found for the IgA antibodies. The ELISA measurements did not reveal any difference in the levels of the various antibody classes between carriers and noncarriers 6 weeks after vaccination (18). Thus, the positive relationship observed before vaccination between high antibody levels and distinct immunoreactive bands for all antibody classes only held for the IgA antibodies after vaccination.

ACKNOWLEDGMENTS

The mouse monoclonal antibody 3-1-P1.2 was a gift from W. D. Zollinger, Walter Reed Army Institute of Research, Washington, D.C., and the peroxidase-conjugated sheep anti-mouse immunoglobulin was a gift from T. E. Michaelsen, National Institute of Public Health, Oslo, Norway. E. Rosenqvist, National Institute of Public Health, provided the ELISA data on the antibody levels of the individual sera. Karin Bolstad performed the silver staining of the SDS-gels and most of the darkroom work.

LITERATURE CITED

1. **Cannon, J. G., W. J. Black, I. Nachamkin, and P. W. Stewart.** 1984. Monoclonal antibody that recognizes an outer membrane antigen common to the pathogenic *Neisseria* species but not to most nonpathogenic *Neisseria* species. Infect. Immun. **43:** 994–999.
2. **Clamp, J. R., and L. Hough.** 1965. The periodate oxidation of amino acids with reference to studies on glycoproteins. Biochem. J. **94:**17–24.
3. **Fazekas de St. Groth, S., R. G. Webster, and A. Datyner.** 1963. Two new staining procedures for quantitative estimation of proteins on electrophoretic strips. Biochim. Biophys. Acta **71:**377–391.
4. **Frasch, C. E., and E. C. Gotschlich.** 1974. An outer membrane protein of *Neisseria meningitidis* group B responsible for serotype specificity. J. Exp. Med. **140:**87–104.
5. **Frasch, C. E., and M. S. Peppler.** 1982. Protection against group B *Neisseria meningitidis* disease: preparation of soluble protein and protein-polysaccharide immunogens. Infect. Immun. **37:**271–280.
6. **Frøholm, L. O., B. P. Berdal, K. Bøvre, P. Gaustad, A. Harboe, E. Holten, E. A. Høiby, A. Lystad, T. O. Omland, and C. E. Frasch.** 1983. Meningococcal group B vaccine trial in Norway 1981–1982. Natl. Inst. Public Health Ann. **6:**133–138.
7. **Gershoni, J. M., and G. E. Palade.** 1982. Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to a positively charged membrane filter. Anal. Biochem. **124:**396–405.
8. **Griffiths, E., P. Stevenson, R. Thorpe, and H. Chart.** 1985. Naturally occurring antibodies in human sera that react with the iron-regulated outer membrane proteins of *Escherichia coli*. Infect. Immun. **47:**808–813.
9. **Hitchcock, P. J.** 1984. Analyses of gonococcal lipopolysaccharides in whole-cell lysates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis: stable association of lipopolysaccharide with the major outer membrane protein (protein I) of *Neisseria gonorrhoeae*. Infect. Immun. **46:**202–212.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **277:**680–685.
11. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
12. **Mandrell, R. E., and W. D. Zollinger.** 1984. Use of a zwitterionic detergent for restoration of the antibody-binding capacity of electroblotted meningococcal outer membrane proteins. J. Immunol. Methods **67:**1–11.
13. **Nicolas, R. H., and G. H. Goodwin.** 1982. Isolation and analysis, p. 41–68. *In* E. W. Johns (ed.), The HMG chromosomal proteins. Academic Press, Inc., London.
14. **Orstavik, K. H.** 1981. Alloantibodies to factor IX in haemophilia B characterized by crossed immunoelectrophoresis and enzyme-conjugated antisera to human immunoglobulins. Br. J. Haematol. **48:**15–23.
15. **Poolman, J. T., S. de Marie, and H. C. Zanen.** 1980. Variability of low-molecular-weight, heat-modifiable outer membrane proteins of *Neisseria meningitidis*. Infect. Immun. **30:**642–648.
16. **Poolman, J. T., C. T. P. Hopman, and H. C. Zanen.** 1983. Immunogenicity of meningococcal antigens as detected in patient sera. Infect. Immun. **40:**398–406.
17. **Poxton, I. R., G. T. Bell, and G. R. Barclay.** 1985. The association on SDS-polyacrylamide gels of lipopolysaccharide and outer membrane proteins of *Pseudomonas aeruginosa* as revealed by monoclonal antibodies and Western blotting. FEMS Microbiol. Lett. **27:**247–257.
18. **Rosenqvist, E., T. Tjade, L. O. Frøholm, and C. E. Frasch.** 1983. An ELISA study of the antibody response after vaccination with a combined meningococcal group B polysaccharide and serotype 2 outer membrane protein vaccine. Natl. Inst. Public Health Ann. **6:**139–149.
19. **Spiro, R. G.** 1966. Characterization of carbohydrate units of glycoproteins. Methods Enzymol. **8:**26–52.
20. **Sugasawara, R. J.** 1985. Recognition of serogroup A *Neisseria meningitidis* serotype antigens by human antisera. Infect. Immun. **48:**23–28.
21. **Towbin, H., and J. Gordon.** 1984. Immunoblotting and dot immunobinding. Current status and outlook. J. Immunol. Methods **72:**313–340.
22. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
23. **Tsai, C. M., R. Boykins, and C. E. Frasch.** 1983. Heterogeneity and variation among *Neisseria meningitidis* lipopolysaccharides. J. Bacteriol. **155:**489–504.

Corteva Exhibit 2037 - Page 13 of 14
Bayer v. Corteva - IPR2023-01036

24. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119:**115–119.
25. **Tsai, C. M., C. E. Frasch, and L. F. Mocca.** 1981. Five structural classes of major outer membrane proteins in *Neisseria meningitidis*. J. Bacteriol. **146:**69–78.
26. **Zollinger, W. D., and R. E. Mandrell.** 1983. Studies of the human antibody response to specific meningococcal outer membrane proteins of serotypes 2 and 15. Med. Trop. (Marseille) **43:**143–147.
27. **Zollinger, W. D., E. E. Moran, H. Connelly, R. E. Mandrell, and B. Brandt.** 1984. Monoclonal antibodies to serotype 2 and serotype 15 outer membrane proteins of *Neisseria meningitidis* and their use in serotyping. Infect. Immun. **46:**260–266.

Corteva Exhibit 2037 - Page 14 of 14
Bayer v. Corteva - IPR2023-01036