INFECTION AND IMMUNITY, Oct. 2001, p. 6044–6054 Vol. 69, No. 10
0019-9567/01/$04.00+0 DOI: 10.1128/IAI.69.10.6044–6054.2001
Copyright © 2001, American Society for Microbiology. All Rights Reserved.

# Molecular Cloning of a *Bacteroides caccae* TonB-Linked Outer Membrane Protein Identified by an Inflammatory Bowel Disease Marker Antibody

BO WEI,[1] HARNISHA DALWADI,[1] LYNN K. GORDON,[2] CAROL LANDERS,[3] DAVID BRUCKNER,[1] STEPHAN R. TARGAN,[3,4] AND JONATHAN BRAUN[1,5]*

*Departments of Pathology and Laboratory Medicine,[1] Ophthalmology,[2] and Medicine[4] and Molecular Biology Institute,[5] University of California, Los Angeles, California 90095, and Inflammatory Bowel Disease Research Center, Cedars-Sinai Medical Center, Los Angeles, California 90048[3]*

Received 13 October 2000/Returned for modification 7 December 2000/Accepted 10 July 2001

**Commensal enteric bacteria are a required pathogenic factor in inflammatory bowel disease (IBD), but the identity of the pertinent bacterial species is unresolved. Using an IBD-associated pANCA monoclonal antibody, a 100-kDa protein was recently characterized from an IBD clinical isolate of *Bacteroides caccae* (p2Lc3). In this study, consensus oligonucleotides were designed from 100-kDa peptides and used to identify a single-copy gene from the p2Lc3 genome. Sequence analysis of the genomic clone revealed a 2,844-bp (948 amino acid) open reading frame encoding features typical of the TonB-linked outer membrane protein family. This gene, termed *ompW,* was detected by Southern analysis only in *B. caccae* and was absent in other species of *Bacteroides* and gram-negative coliforms. The closest homologues of OmpW included the outer membrane proteins SusC of *Bacteroides thetaiotaomicron* and RagA of *Porphyromonas gingivalis*. Recombinant OmpW protein was immunoreactive with the monoclonal antibody, and serum anti-OmpW immunoglobulin A levels were elevated in a Crohn's disease patient subset. These findings suggest that OmpW may be a target of the IBD-associated immune response and reveal its structural relationship to a bacterial virulence factor of *P. gingivalis* and periodontal disease.**

Human inflammatory bowel disease (IBD) represents a set of a chronic, relapsing, and remitting intestinal inflammatory disorders involving T-cell-mediated mucosal and mural destruction and polygenic familial susceptibility (34, 35). Several spontaneous and transgenic mouse strains have been established with susceptibility for chronic colitis similar to human IBD (4, 5, 18, 20, 26, 32). In all evaluated models, normal resident enteric bacteria were found to be required for disease pathogenesis (13, 27, 40). Similarly, in human IBD several lines of evidence implicate enteric bacteria as a pathogenic factor in clinical disease, particularly in Crohn's disease (CD) (3, 33, 48).

Immunologic studies have demonstrated that antibody and T-cell reactivity to commensal enteric bacteria is a distinguishing feature of colitic mouse strains (6, 10). However, the bacterial species and antigens recognized by colitigenic T cells have not yet been defined. Moreover, monoassociation studies have not yet revealed pathogenic bacterial species for colitis-prone mouse strains (27). A systematic approach to this issue is hampered by the limited understanding of gastrointestinal microflora ecosystem. In addition, immune recognition of this commensal microflora in normal individuals is attenuated or undetectable. Accordingly, it has been difficult to highlight bacterial species or antigens for evaluation in IBD pathogenesis.

Marker antibodies have been used successfully to identify disease-relevant antigenic targets in several immune-mediated diseases (38, 45). In IBD, approximately 60 to 70% of ulcerative colitis (UC) patients and 25% of CD patients have elevated levels of pANCA, an antineutrophil cytoplasmic antibody with distinctive morphological and antigenic fine specificity (15, 30, 36, 41, 51). An immunochemical study has associated pANCA with enteric bacterial antigens by serum cross-reactivity in human and mouse (39). This observation provides a precedent for the hypothesis that pANCA antibody would identify the antigenic proteins responsible for the pathogenic mucosal inflammation.

We have addressed this hypothesis using two pANCA monoclonal antibodies (Fab 5-3 and 5-2) to search for bacterial cross-reactive proteins associated with the UC-specific immune response. These antibodies were isolated by phage display technology from lamina propria lymphocytes of UC patient. Their concordance with serum pANCAs was validated by the criteria of immunofluorescence, confocal microscopy, and DNase I sensitivity (17). To our knowledge, these are the only reported pANCA monoclonal antibodies. Experience has shown that antigen discovery with individual monoclonal antibodies can be misleading and is best pursued with a diverse monoclonal antibody panel. However, because of the unique disease association of pANCA, we proceeded with the available monoclonal antibodies in a bacterial antigen search.

Using Fab 5-3, we identified two candidate bacterial pANCA antigens in a search of colonic bacterial clinical isolates from an IBD patient: OmpC of *Escherichia coli*, and a novel 100-kDa protein of *Bacteroides caccae* (8). In the present study, the gene encoding the 100-kDa protein is cloned and characterized. We show that this protein is a new TonB-linked outer membrane protein (termed OmpW) closely related to the RagA virulence factor of *Porphyromonas gingivalis*. Immunologic assessment demonstrated that immunoglobulin A (IgA) anti-OmpW is elevated in a subset of CD patients. These

* Corresponding author. Mailing address: Department of Pathology and Lab Medicine, UCLA School of Medicine, CHS 13–222, Los Angeles, CA 90095-1732. Phone: (310) 794-7953. Fax: (310) 825-5674. E-mail: jbraun@mednet.ucla.edu.

finding suggest that *B. caccae* and OmpW may be bacterial targets of the disease-related immune response in IBD.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** A panel of *Bacteroides* clinical isolates (3955-3, 4536, 4552, 4556, 4562, 4570, 4578, and 4579) were stored and cultured in the clinical laboratories at University of California, Los Angeles (UCLA). *B. vulgatus* strains LG-1, LG1-33, and CPT-6 were kind gifts from R. B. Sartor, University of North Carolina at Chapel Hill. *B. caccae* strains 43185 and p2Lc3 were from the American Type Culture Collection and a colonic isolate of a Crohn's disease patient (8), respectively. Clinical isolates of *E. coli*, *Salmonella enterica* serovar Typhi, and *Shigella flexneri* were provided by UCLA Clinical Laboratories. All *Bacteroides* strains were grown on brucella blood agar in an anaerobic chamber with 10% $CO_2$–90% $N_2$ atmosphere at 37°C.

**Recombinant cloning reagents.** *E. coli* XL-1 Blue strain (Stratagene, La Jolla, Calif.) was used for all cloning and recombinant expression experiments. The pBluescript vector (Stratagene) and pCR 2.1 plasmid (Invitrogen, Carlsbad, Calif.) vector were used for cloning in *E. coli* XL-1 Blue and selected on Luria-Bertani (LB) medium agar plate (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1.5% agar) supplemented with ampicillin (100 μg/ml). Blue or white colony color selection was used to distinguish between nonrecombinant and recombinant *E. coli* clones by spreading X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and IPTG (isopropyl-β-D-thiogalactopyranoside) on LB plates in the cloning procedures. All restriction enzymes used in this study were purchased from New England Biolabs (Beverly, Mass.). Human monoclonal anti-pANCA antibody (Fab 5-3) was used to analyze recombinant OmpW protein (17). Alkaline phosphatase-conjugated goat anti-human $F(ab)_2$ (Pierce, Rockford, Ill.) was used as the secondary antibody in Western blot analysis.

**Construction and screening of genomic library.** Genomic library of a *B. caccae* clinical isolate (p2Lc3) was constructed using lambda ZAPII λ phage vector (Stratagene, La Jolla, Calif.). Briefly, chromosomal DNA was purified from p2Lc3 using the ASAP genomic DNA isolation kit (Boehringer Mannheim, Indianapolis, Ind.), partially digested with *Eco*RI, and fragments ranging from 1.5 to 9 kb were ligated into lambda ZAPII *Eco*RI-digested, calf intestinal alkaline phosphatase (CIAP)-treated vector. Recombinant lambda phages were packaged, and the PFU of packaging reaction (primary library) were titrated. The primary library was plated at appropriate dilution on large 150-mm agar plates to $5 \times 10^4$ PFU/plate and incubated at 37°C for 8 h. The phage plaques from each plate were transferred onto duplicated Hybond-N membranes (Amersham Pharmacia Biotech, Piscataway, N.J.), denatured in 0.5 N NaOH–1.5 M NaCl, and neutralized in 0.5 M Tris-Cl–1.5 M NaCl (pH 8.0), rinsed in 0.2 M Tris-Cl–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) buffer solution, and fixed using the Stratalinker UV cross-linker (Stratagene).

According to *Bacteroides* preferred codons, two oligonucleotide probes were designed for the 100-kDa N-terminal peptides DPSSLAIFGVR (Pep1) and GPSEADAFYNC (Pep2) and purchased from Gibco-BRL (Rockville, Md.). The oligonucleotides were labeled with $^{32}P$ by T4 polynucleotide kinase using DNA 5′-end labeling kit (Boehringer Mannheim, Indianapolis, Ind.), and used to screen p2Lc3 genomic libraries. The same probes were also used to identify positive phagemids in Southern blot analysis.

The membranes were prehybridized in Rapid-Hyb buffer (Amersham Pharmacia) for at least 1 h and then hybridized with a mixture of [$^{32}P$]Pep1 and [$^{32}P$]Pep2 probes in the same buffer for 6 h under low-stringency conditions (40°C). Positive plaques in primary screening were subjected to secondary screening using the same conditions. Following the secondary screening on the positive clones, in vivo excision of the positive phage isolates was performed to obtain the insert-containing pBluescript phagemid as described in the manufacturer's instruction.

**Cloning and sequencing the full-length 100-kDa protein gene (OmpW).** Based on primary sequence information obtained from the original genomic library clones, genome-walking strategy (rapid amplification of cDNA ends [RACE]) was employed to clone full-length gene encoding 100-kDa protein using Universal GenomeWalker kit (Clontech Laboratories, Palo Alto, Calif.). Reverse GSP and Forward GSP oligonucleotide pairs were designed to obtain upstream and downstream flanking sequences of the primary gene fragment, respectively (Table 1).

For DNA sequencing, plasmid DNA samples were prepared using Qiafilter Plasmid Kit (Qiagen, Valencia, Calif.). DNA sequence analysis was performed at the University of California–Los Angeles Sequencing Facility using dideoxy dye termination PCR. Complete coverage of entire gene sequence and linkage of each contiguous fragment were achieved by subcloning and primer walking. Both strands were completely sequenced and assembled using the Wisconsin Genetics Computer Group (GCG) sequence analysis programs. The OmpW sequence was characterized using the BLASTX program (version 20.11, 20 January 2000) at National Center for Biotechnology Information and National Institutes of Health nonredundant databases. The analysis of amino acid sequence alignments was performed using ClustalW multiple-sequence alignment program at EMBL Outstation European Bioinformatics Institute and displayed by the GenDoc program (www.psc.edu/biomed/genedoc).

TABLE 1. ompW oligonucleotides

| Name | Sequence (5′→3′) |
|---|---|
| Pep1 | GACCCGTCTTCCCTGGCTATCTTCGGTGTTCGT |
| Pep2 | GGTCCGTCCGAAGCTGACGCTTTCTACAACTG |
| Reverse GSP1 | TGCCACTATGCTCTTAAACCAAGTGGTTTCTTCT |
| Reverse GSP2 | CGCTATTTGGCGAAAGTACAAAGTGAA |
| Forward GSP1 | GACGGTTCTTCCGCTTTCTCTTATACG |
| Forward GSP2 | CAACGAATGGCAGAACTTCTTCTCTCT |
| OmpW-f | ACGTATGCTTCCCGCCGACTCCAAACAAGT |
| OmpW-r | AGTCGTGAAACCTGCAGTAGCAGTCAGGT |
| OmpW-1f | ATTGGATCCATGGACGGTAAGTATTCAATCTCTGTC |
| OmpW-2f | ATCGGATCCATGGAGATTCTGAAAGACCCGTCTTCA |
| OmpW-3f | ATGGATCCATGCTTCCCGCCGACTCCAAACAAGTA |
| OmpW-R | TATGAGCTCATTAGAATGTCAGGTTGATACCG |
| 16S-f | CCTACGGGAGGCAGCAG |
| 16S-r | ATTACCGCGGCTGCTGG |

**OmpW-specific PCR.** To test *ompW* distribution in enteric bacterial strains, PCR was performed on bacterial chromosomal DNA samples to amplify an *ompW*-specific amplicon of 468 bp. Chromosomal DNA was extracted from bacterial strains and used as the templates in the PCR analysis with *ompW*-specific primers (OmpW-f and OmpW-r) spanning nucleotides 893 to 1361 of the open reading frame (Table 1). A phylogenetically conserved segment of the bacterial 16S RNA was used to quantitate bacteria DNA sample in PCR (16S primers, Table 1) (31). The PCRs were performed in a 50-μl volume consisting of 1 μg of genomic DNA, 0.5 U of *Taq* polymerase, 2 mM deoxynucleotide triphosphate mixture, and 1 μM each of primers in 1× PCR buffer using Gene-Amp PCR System 9700 (PE Applied Biosystems): 5 min at 95°C followed by 30 cycles of 95°C for 60 s, 65°C for 60 s, and 72°C for 60 s. After the final cycle, the reaction was extended at 72°C for 5 min and cooled at 4°C.

**Southern blot analysis.** Southern blot analysis was employed to identify positive phages screened from genomic library and to analyze *ompW* gene distribution in bacterial clinical isolates. To identify insert-containing phagemids, digested plasmid DNAs were electrophoresed on 0.8% agarose gel and depurinated in 0.5 M HCl, denatured in 0.5 M Tris-Cl–1.5 M NaOH, and neutralized in 0.5 M Tris-Cl–1.5 M NaCl buffer, and then transferred onto Hybond N+ membrane (Amersham) by capillary blotting. After blotting, the membranes were prehybridized for at least 1 h at 60°C in Rapid-Hyb buffer (Amersham Life Sciences) and hybridized at 60°C for 6 h in the same buffer with a mixture of $^{32}P$-labeled Pep1 and Pep2 oligonucleotide probes. Membranes were washed for 15 min three times with 2× SSC containing 0.1% SDS at room temperature and then washed with 0.1× SSC containing 0.1% sodium dodecyl sulfate (SDS) at 60°C for 15 min three times. Autoradiography was carried out at −70°C with intensifying screens for an optimized exposure time on Hyper Film (Amersham Life Sciences).

To analyze the phylogeny of *ompW*, chromosomal DNAs of different bacterial strains were purified using GenomePrep DNA isolation kit (AmershamPharmacia); 5 μg of purified DNA samples were digested by *Hin*dIII, electrophoresed, and transferred to membranes. A 0.7-kb insert in pBS 0.7 plasmid, which is located from site 369 to site 1029 in full-length *ompW* gene, was used as the probe for hybridization with restricted bacterial genomic DNAs. The *ompW*-specific probe was labeled with $^{32}P$ by the random-primer method using Prime It II system (Stratagene). The hybridization was performed at 65°C in Rapid-Hyb buffer for 3 to 6 h (Amersham Life Sciences). The membranes were treated as described above and exposed to Hyper Film at −70°C.

**Expression of recombinant OmpW protein.** To express the OmpW proteins, pairs of primers were designed to clone the full-length and truncated OmpW open reading frame segments directly from p2Lc3 genome by PCR. Forward primers used to amplify the full-length ORF (OmpW1) and the OmpW2 and OmpW3 truncated proteins were designated OmpW 1f, 2f, and 3f, respectively. OmpW-R was used as the reverse primer for cloning three segments (Table 1). In order to facilitate the construction of expression vectors, a *Bam*HI site was designed in the forward primers and a *Sac*I site in reverse primer for each

fragment. The PCR products were inserted in frame into His-taggged expression vector pQE-30 (Qiagen). The full-length and truncated OmpW proteins were expressed in *E. coli* XL-1 Blue strain as 6× His-tagged proteins and purified by HisTrap column (AmershamPharmacia) under denatured conditions according to manufacturer's instructions.

**Western blot analysis.** The full-length and truncated recombinant OmpW proteins were quantified using the Bradford assay (Bio-Rad Laboratories, Hercules, Calif.), and equivalent protein amounts (2 μg/well and 0.5 μg/well) were separated on 12% polyacrylamide gels under reducing conditions. Electrophoresed proteins were transferred overnight onto nitrocellulose membranes (Amersham Life Sciences) in Tris-glycine buffer (National Diagnostics, Atlanta, Ga.) and verified by Ponceau S red staining (Sigma Chemicals, St. Louis, Mo.) or Coomassie blue staining. Membranes were blocked in 5% nonfat milk (Carnation, Glendale, Calif.) in PBS with 0.1% Tween-20 (PBS-Tween) for 1 h. Fab 5-3 antibody diluted in 1% milk–PBS–Tween was incubated with membranes for 1 h. Immunoblots were detected by alkaline phosphatase-conjugated goat anti-human $F(ab)_2$ and developed with 5-bromo-4-chloro-3-indolylphosphate–nitro blue tetrazolium (BCIP/NBT) liquid substrate system (Sigma Chemicals).

**OmpW serum ELISA.** Human antibodies that bind OmpW were detected by enzyme-linked immunosorbent assay (ELISA). Plates (USA Scientific, Ocala, Fla.) were coated overnight at 4°C with 100 μl/well of OmpW recombinant protein at 5 μg/ml in borate-buffered saline, pH 8.5. After three washes in 0.05% Tween 20 in PBS, the plates were blocked with 150 μl/well of 0.5% bovine serum albumin in PBS, pH 7.4 (BSA-PBS), for 30 min at room temperature (RT) and washed again prior to incubation with sera. Then 100 μl/well of serum from CD patients, UC patients and normal controls at various dilutions were added in duplicate and incubated for 2 h at RT. The plates were washed and incubated with alkaline phosphatase-conjugated goat anti-human IgA or anti-human IgG (Jackson ImmunoResearch, West Grove, Pa.) at a dilution of 1:1,000 in BSA-PBS for 2 h at RT. The plates were washed three times with 0.05% Tween 20 in PBS followed by another three washes with Tris-buffered normal saline, pH 7.5. Substrate solution (1.5 mg/ml of disodium *P*-nitrophenol phosphate; Amresco, Solon, Ohio) in 2.5 mM $MgCl_2$–0.01 M Tris, pH 8.6, was added at 100 μl/well to allow color development. The absorbances were measured at 405 nm. Values for pANCA activity was determined by neutrophil ELISA and categorized by neutrophil immunofluorescence, as previously described (37).

Quantitative data were compared using the Mann-Whitney test. This nonparametric statistic was selected because, with the relatively small sample size, Gaussian distribution of the group data sets could not be established. Contingency table data sets were analyzed with Fisher's exact test. This test was selected because it calculates *P* values exactly and is a valid method for analyzing small absolute numbers of cells. Statistical analysis was performed with Prism 3.0 (GraphPad Software, San Diego, Calif.).

**Human subjects.** Serum samples from 69 subjects (23 each of UC patients, CD patients, and healthy controls) were obtained from the serum archive of the Cedars-Sinai IBD Research Center. Sera were produced from standard phlebotomy blood specimens, anonymously number coded, aliquoted, and stored at −80°C until use. The UC and CD patient specimens were drawn from an ongoing genetic case-control study; the demography, disease ascertainment methods, disease activity, and treatment profile of this population have been described previously (47, 51). At the time of our analysis, this study contained 240 UC and 213 CD probands, respectively. Each patient was diagnostically validated by clinical history, endoscopic and radiologic examination, and histopathology findings.

Sera from this archive were selected by a simple concurrent control method. Specimens accrued during 1999 were accessed, and 23 CD and UC sera were chosen solely on the basis of maximum residual volumes. Due to the relatively small size of the specimen set used in this study, the data were not stratified for clinical features (disease duration, disease activity, and treatment profile). Anti-OmpW levels were measured in this study; the pANCA levels of these sera were tabulated from archive data. The latter data revealed that the 23 UC and CD specimens included 12 and 7 pANCA-positive specimens, respectively. Normal controls were from concurrent blood bank donations with known age, ethnicity, and blood type.

Procedures for subject recruitment, informed consent, and specimen procurement were in accordance with protocols approved by the Institutional Human Subject Protection Committees of UCLA and Cedars-Sinai Medical Center.

**Nucleotide sequence accession number.** The nucleotide sequence data for the *ompW* gene of *B. caccae* reported in this study have been assigned GenBank accession number AF305878.

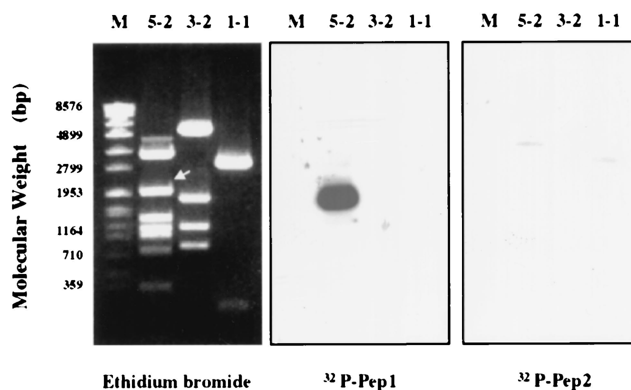


FIG. 1. Pep1 hybridization of a 2-kb AccI genomic segment of *B. caccae*. Three clones (5-2, 3-2, and 1-1) were isolated from a primary screen of a genomic *B. caccae* λ phage library. The clones were digested with *Acc*I, electrophoresed, transferred to nitrocellulose membranes, and hybridized with the Pep1 or Pep2 oligonucleotide probe. (Left panel) Ethidium bromide-stained gel; (middle panel) membrane hybridization with Pep1 oligonucleotide; (right panel) membrane hybridization with Pep2 oligonucleotide.

## RESULTS

**Cloning of full-length gene encoding 100-kDa protein of *B. caccae*.** The first goal of this study was to clone the gene encoding 100-kDa protein of *B. caccae*. A genomic library of p2Lc3 was constructed by ligating *Eco*RI-restricted chromosomal DNA into ZAPII phage vector. Two oligonucleotide probes were designed using *Bacteroides* preferred codons from two N-terminal peptide sequences (Pep1 and Pep2) of the tryptic 100-kDa protein (8). To ensure a positive result in library screening, a mixture of $^{32}$P-labeled Pep1 and Pep2 probes was used to hybridize with phage library under low-stringency conditions (40°C). Approximately $10^6$ phage plaques of the library were screened, and eight positive clones were identified.

Following the secondary screening on the positive clones, in vivo excision on the isolates was carried out, and three insert-containing phagemids were obtained (designated 3-1, 3-2, and 5-2). The inserts in the three phagemids were 0.2 kb, 6.9 kb, and 7.2 kb. To further localize the probe-identified sequence in the insert, restriction enzyme-digested phagemids were hybridized with Pep1 and Pep2 probes separately in Southern blot analysis. This revealed a 2-kb *Acc*I fragment in 5-2 genomic clone which hybridized strongly with the Pep1 probe (Fig. 1). Initial sequence analysis of the 2-kb fragment and its flanking sequence indicated that the insert in phagemid 5-2 was interrupted by a 1.2-kb Tn*10* transposon sequence. However, PCR analysis of p2Lc3 genomic DNA showed that this transposon insertion was absent in the *B. caccae* genome and presumably was the result of an artifactual transposon insertion during cloning manipulation (data not shown).

In order to obtain authentic flanking sequences of 2-kb fragment, a genome walking strategy was employed to clone full-length *ompW* gene directly from the p2Lc3 genome. RACE cloning produced 0.9-kb upstream and 1.3-kb downstream sequences flanking the p2Lc3 2-kb fragment. In total, 4,034 bp were cloned and sequenced by this process.

**Sequence analysis and structural features of deduced OmpW protein.** The major cloning steps and the structural features of the OmpW genomic segment are summarized in Fig. 2. The

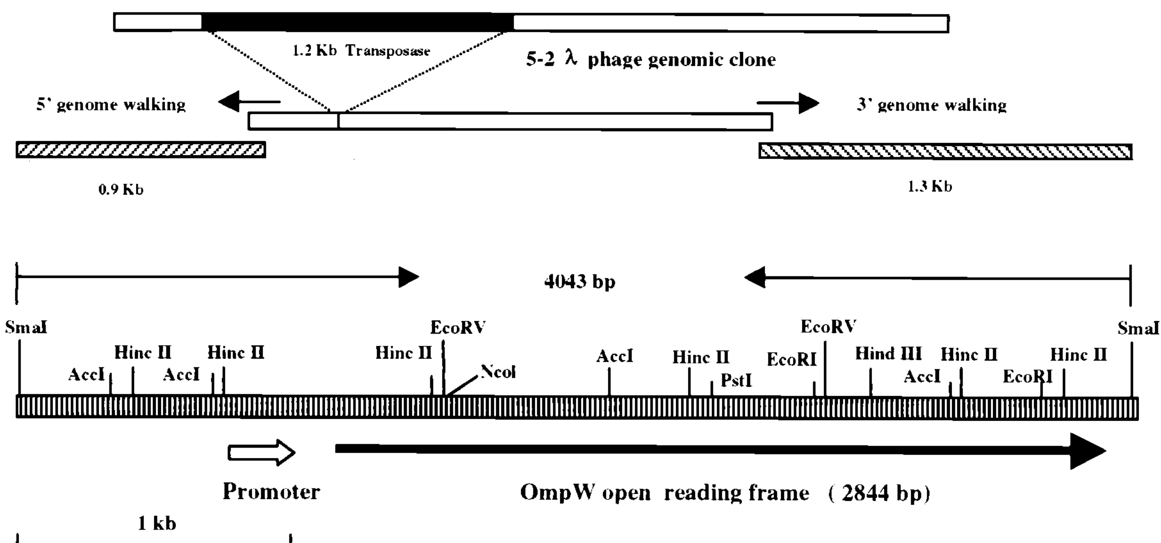


FIG. 2. Cloning of the *ompW* gene. A genomic λ phage library of p2Lc3 was constructed and screened using Pep1 and Pep2 probes. Southern analysis identified an authentic Pep1-positive genomic clone, designated 5-2 (Fig. 1), which contained an artifactual insert Tn*10* sequence. The native *ompW* gene was isolated by genome walking using p2Lc3 genomic DNA as substrate. Sequence analysis of a 4,043-bp fragment revealed an open reading frame encoding 947 amino acids and an upstream AT-rich putative promoter locus.

nucleotide sequence analysis of 4,034-bp p2Lc3 genomic fragment revealed a 2,844-bp-long open reading frame (ORF) encoding a putative outer membrane protein with a predicted molecular mass of 105 kDa and isoelectric point of 5.96. The Pep1 sequence was identified within this ORF. A 189-bp AT-rich region was identified upstream of the ORF (G+C content of 34%, compared to 48% for the *ompW* ORF), perhaps representing a promoter region. No homologies to known prokaryotic promoter sequences were found in this putative *ompW* promoter region. However, this might reflect the structural divergence of regulatory elements in *Bacteroides* compared to the more commonly characterized *E. coli* elements (43).

Nucleic acid and amino acid homology analysis (BlastN and BlastX) of the NCBI databases indicated that OmpW was homologous to the outer membrane proteins SusC of *Bacteroides thetaiotaomicron* and RagA of *Porphyromonas gingivalis* (Fig. 3). The amino acid similarity of OmpW was comparable to SusC (identity, 30%; similarity, 47%) and RagA (identity, 29%; similarity, 46%). The two regions of greatest similarity were located in the N-terminal 270 residues and the extreme C terminus.

The deduced OmpW protein had features typical of bacterial TonB-linked outer membrane proteins. A multiple sequence alignment comparing OmpW with SusC, RagA, and outer TonB-linked receptors for iron acquisition or vitamin uptake from various bacterial species is shown in Fig. 4. The TonB box (amino acids 160 to 193) is highly conserved among TonB-dependent outer membrane receptors and is present in OmpW. Other more N-terminal (87 to 96, 109 to 113, and 126 to 131) clusters of homology were also observed. The C-terminal segment of OmpW also exhibits similarity to TonB-dependent receptor protein: a C-terminal phenylalanine residue and the hydrophobic amino acids at positions −3, −5, −7, and −9. The same residues are found in other TonB-linked outer membrane proteins and are thought to have important function in outer membrane protein assembly and sorting (44, 46).

**OmpW is specific to *B. caccae*.** To determine the species selectivity of the OmpW, the distribution of the *ompW* gene in different *Bacteroides* strains and other gram-negative coliforms (*E. coli, Salmonella enterica* serovar Typhi, and *Shigella flexneri*) was analyzed by PCR and Southern blot. PCR was performed on chromosome DNAs of the enteric bacterial strains (Fig. 5). The 468-bp *ompW*-specific sequence was detectable in both available *B. caccae* isolates, but was undetectable among four *Bacteroides* species and eight isolates, and the *E. coli*, *S. enterica* serovar Typhi, and *S. flexneri* strains. In accord with PCR results, only *B. caccae* strains showed a strong hybridizing band by Southern blot analysis, demonstrating that *ompW* is a *B. caccae*-specific gene (Fig. 6). The result also indicated that the gene encoding 100-kDa protein existed partially in a 10-kb *Hin*dIII restriction fragment of the *B. caccae* genome as a single copy.

**The Fab 5-3 pANCA monoclonal antibody recognizes the recombinant OmpW protein.** To characterize the immunological properties of the deduced OmpW protein, three gene fragments encoding full-length and truncated OmpW proteins (as shown in Fig. 7A) were cloned directly from p2Lc3 genome by PCR and inserted in frame into pQE30, a His-tagged protein expression vector. The OmpW1, OmpW2, and OmpW3 proteins (947, 805, and 648 amino acids, respectively) were purified by nickel chromatography, separated on 12% acrylamide gel (Fig. 7B), and evaluated for reactivity to the original Fab 5-3 pANCA monoclonal antibody by Western blot analysis (Fig. 7C). It was indicated that Fab 5-3 recognized OmpW1 and OmpW2, but not OmpW3. These findings confirm that we had cloned the intended Fab5-3 monoclonal antibody-pANCA-reactive *Bacteroides* protein. They also suggest that the Fab5-3 monoclonal antibody-pANCA epitope is located towards the N terminus of the protein, between amino acid positions 143 and 300.

**OmpW is an antigenic target in CD patients.** Purified recombinant OmpW1 and OmpW2 proteins were used for

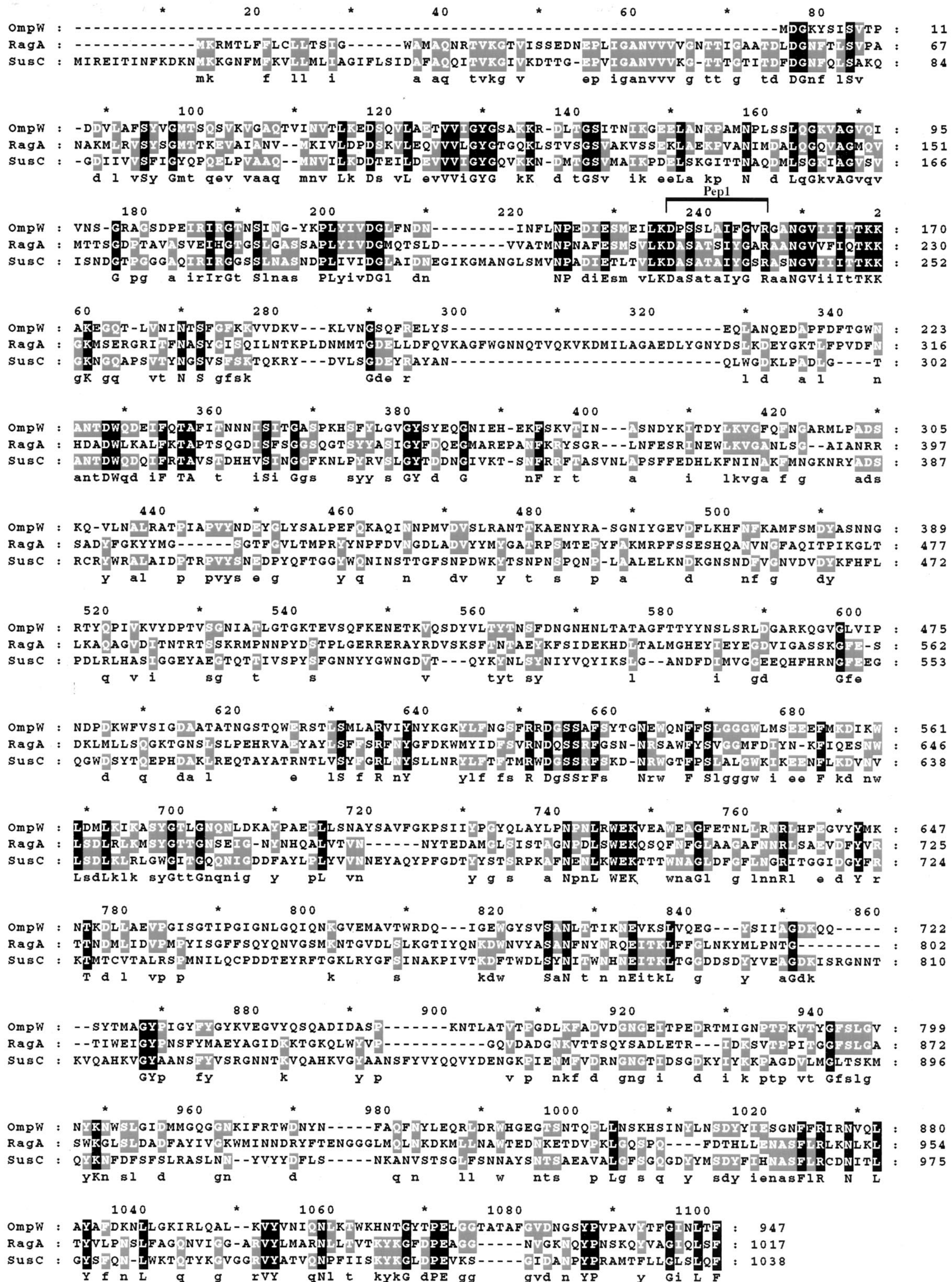


FIG. 3. Sequence homology of OmpW with SusC and Rag A outer membrane proteins. The amino acid sequence of OmpW long open reading frame is aligned with its two closest database homologues, SusC of *B. thetaiotaomicron* and RagA of *P. gingivalis*. The peptide 1 sequence, used as a cloning probe, is also shown. Alignments were performed using the ClustalW multiple-sequence alignment program and are displayed using the GenDoc program. Residues with similarity (identical or conservative amino acid changes) among all sequences are denoted by an uppercase letter, and with single discordances by a lowercase letter or number. Gradations of similarity frequency are denoted by dark to pale shading.

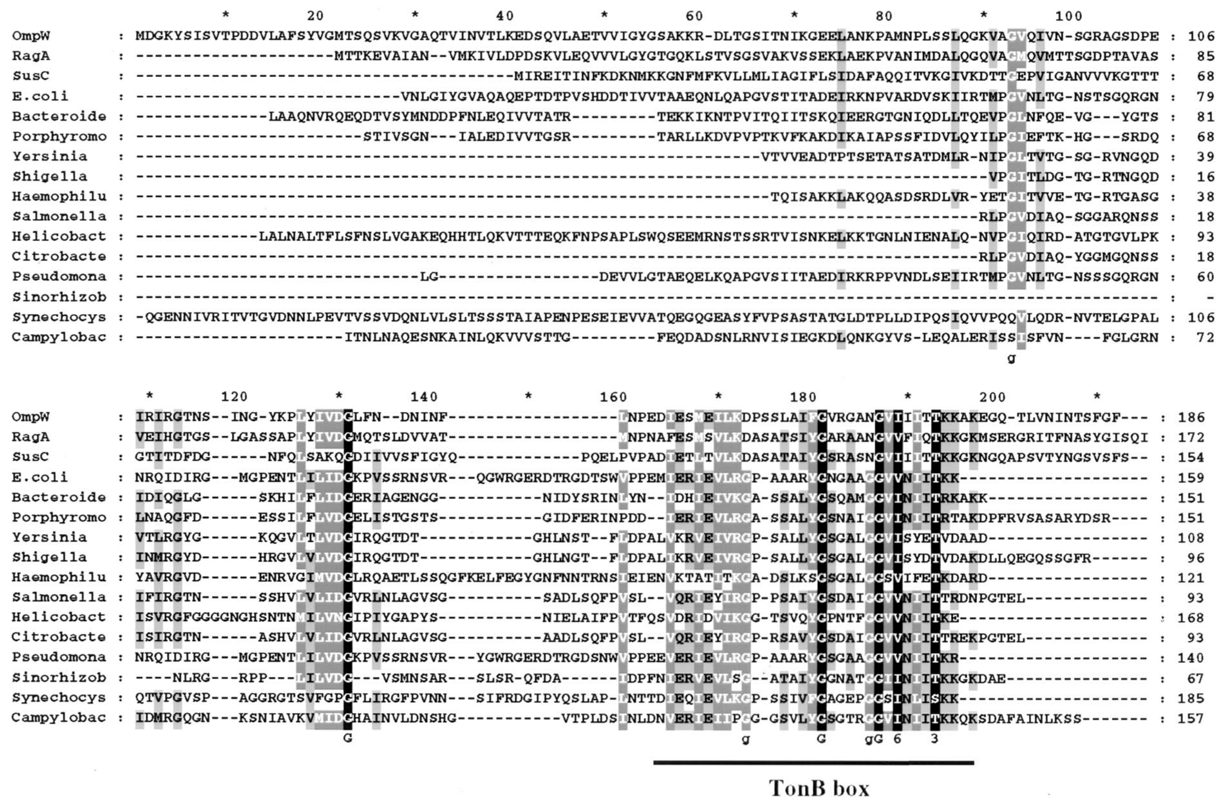


FIG. 4. OmpW contains a bacterial TonB box. Homologous sequences of TonB-linked proteins from different bacterial species were identified by BlastX and aligned and displayed as described in Fig. 3. E.coli, *E. coli*; Porphyromo, *P. gingivalis*; Bacteroide, *Bacteroides fragilis*; Yersinia, *Yersinia enterocolitica*; Shigella, *Shigella dysenteriae*; Haemophilu, *Haemophilus influenzae*; Salmonella: *Salmonella enterica* serovar Typhimurium; Helicobac, *Helicobacter pylori*; Citrobacte, *Citrobacter freundii*; Pseudomona, *Pseudomonas* sp; Synorhizob, *Sinorhizobium meliloti*; Synechocys, *Synechocystis* sp; Campylobac, *Campylobacter jejuni*. The TonB box is labeled.

ELISA evaluation of human anti-OmpW antibody levels in human subjects (Fig. 8). The mean OD units of IgA anti-OmpW were numerically elevated in the CD group (median, 0.168) compared to the UC (0.097) and normal (0.082) groups. Compared to normals, this difference was significant for the CD group ($P < 0.002$) but not the UC group. Similarly, the frequency of positive seroreactivity was elevated in the CD group (35%, 8 of 23), compared to the UC group (13%, 3 of 23) and normal subjects (4%, 1 of 23). The frequency was significantly greater in the CD versus normal group ($P < 0.009$); no significant difference was observed between UC and normals (Fig. 8A). These findings reflect an antibody response which is CD associated but of moderate scale and expressed in a minority of CD patients.

Since the Fab 5-3 antibody reacted with both the full-length and truncated OmpW protein, we compared the levels of IgA anti-OmpW1 and anti-OmpW2. As shown in Fig. 8B, these levels were highly correlated by linear regression ($r^2 = 0.87$, $P < 0.0001$). In contrast to IgA, anti-OmpW2 IgG levels were relatively high in both normal and IBD patients and were not significantly different between groups (Fig. 8C). The mechanism and significance of this isotype restriction remain to be defined.

We further assessed whether IgA anti-OmpW2 was correlated with serum pANCA activity. UC and CD patients were stratified for pANCA positive or negative status by immunofluorescence and DNase antigen sensitivity (2, 37, 49) and compared for IgA anti-OmpW2 mean OD units and seropositivity frequency (Fig. 8D). By both criteria, pANCA status did not correlate with anti-OmpW2 activity. These patient groups were further evaluated for the correlation of quantitative levels of pANCA and IgA anti-OmpW2 (Fig. 8E and F). By linear regression, pANCA and anti-OmpW2 were not significantly correlated for either UC ($r^2 = 0.3$, $P = 0.06$) or CD patients ($r^2 = 0.10$, $P = 0.19$). Most of the anti-OmpW-positive CD patients were pANCA negative. These findings indicate that anti-OmpW is CD associated but may reflect a distinct specificity from the predominant antineutrophil activity in UC patients.

## DISCUSSION

In this study, the *B. caccae* protein identified by an IBD-associated pANCA monoclonal antibody was characterized by molecular cloning and immunologic evaluation of the recombinant gene product. The protein, termed OmpW, was found to be a new member of the TonB-linked outer membrane protein family. OmpW was also closely related to RagA, a virulence factor of the periodontal disease pathogen *P. gingivalis*. Evaluation of patient sera demonstrated increased anti-OmpW IgA levels in CD patients. The issues raised by this study are the relationship of OmpW to the outer membrane protein fam-

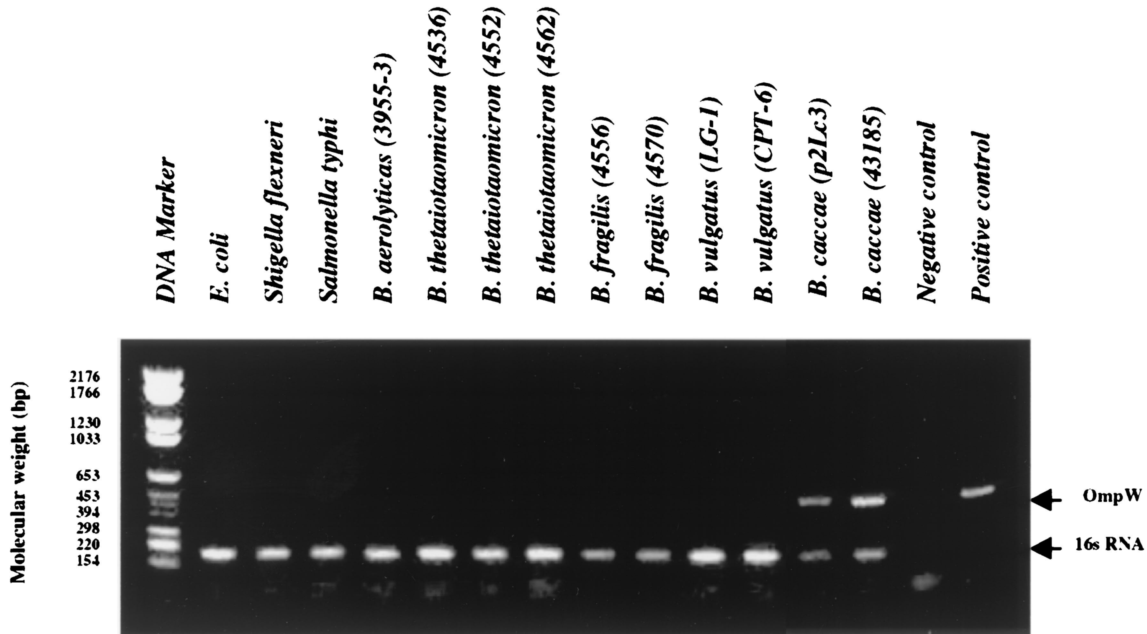


FIG. 5. PCR detection of *ompW* distribution in enteric bacteria. Chromosomal DNA samples from a panel of *Bacteroides* strains and gram-negative coliforms (*E. coli*, *Salmonella enterica* serovar Typhi, *Shigella flexneri*) were detected by PCR for the prevalence of *ompW* gene. The DNA samples were quantitated by the detection of conserved bacterial 16S rRNA with 16S-f and 16S-r primers (Table 1) in the same PCR. Negative control, no template DNA; positive control, *ompW* plasmid clone.

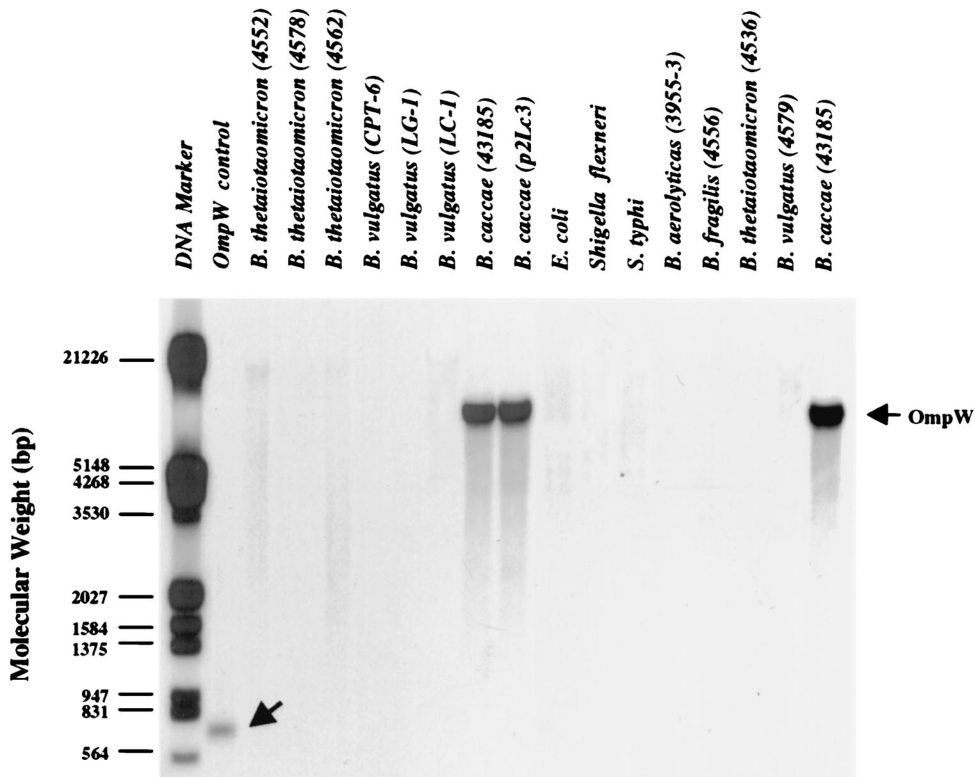


FIG. 6. Southern blot analysis of *ompW* gene distribution in bacterial genomic DNA. A total of 5 μg of the *Hin*dIII-restricted bacterial genomic DNAs were separated on 0.8% agarose gel and transferred onto nitrocellulose membrane. The membranes were hybridized with a mixture of $^{32}$P-labeled *ompW*-specific fragment (0.7 kb) and DNA markers under low-stringency conditions (40°C). The membranes were exposed for an optimized time on Hyper Film. OmpW control, 10 pg of *ompW* restriction fragment.

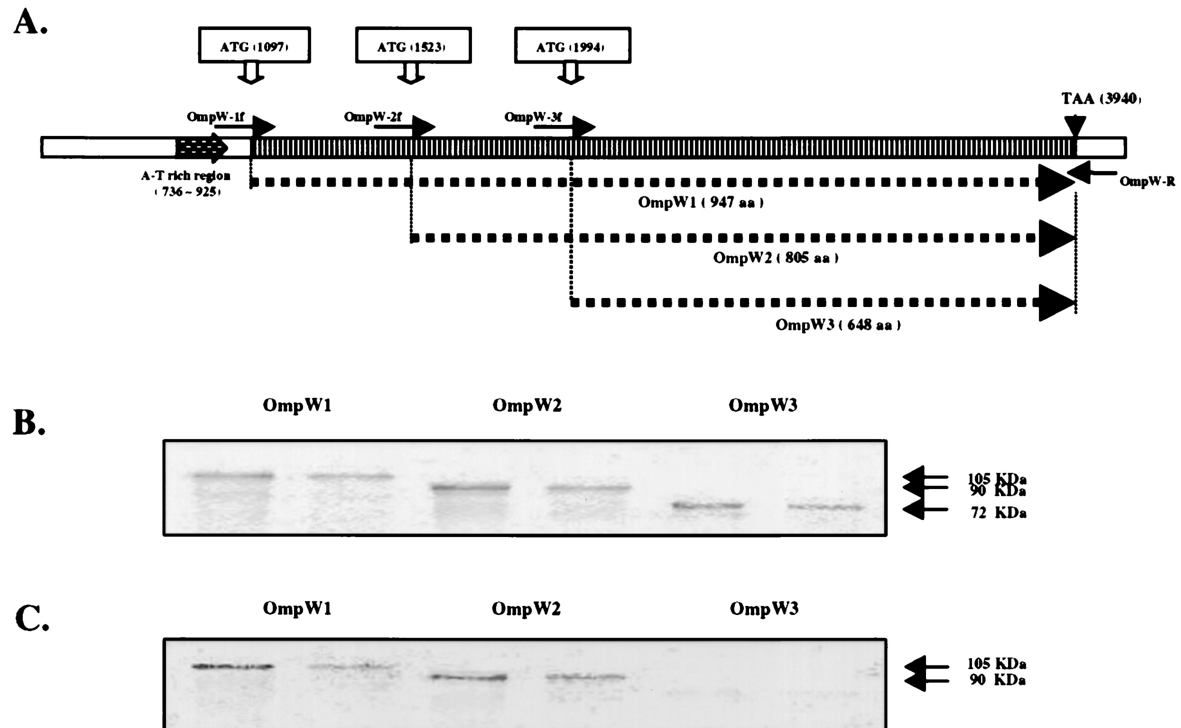


FIG. 7. Expression and analysis of full-length and truncated OmpW proteins. (A) Strategy for expression of OmpW proteins. The gene fragments encoding the full-length and truncated OmpW proteins were cloned directly from p2Lc3. The OmpW-1 ORF starts from the first putative start codon, located at site 1097 in the 4.34-kb cloned fragment. OmpW-2 and OmpW-3 start from positions 1523 and 1994, respectively. The cloned OmpW segments were inserted into vector pQE30, expressed as His-tagged recombinant proteins, and purified by nickel chromatography. (B) SDS-PAGE analysis of purified recombinant OmpW proteins. The purified recombinant OmpW proteins were separated at 2 μg/well and 0.5 μg/well on a 12% acrylamide gel and probed with human pANCA antibody (Fab 5-3) in Western blot analysis. (C) Western blot analysis of expressed OmpW proteins with Fab5-3 pANCA antibody. The recombinant OmpW proteins were probed with Fab5-3 human monoclonal antibody and then detected by alkaline phosphatase-conjugated anti-human $F(ab')_2$ and developed by NCIP/NBT substrate.

ily, its relationship to candidate pANCA antigens, and the role of OmpW and *B. caccae* in IBD pathogenesis.

**Identification of the 100-kDa antigen.** OmpW was cloned from a *B. caccae* genomic library probed with oligonucleotide derived from 100-kDa tryptic peptide sequences. OmpW corresponds to the 100-kDa antigen by several criteria. First, the predicted molecular mass was 105 kDa, and this size was confirmed by SDS-PAGE analysis of recombinantly expressed OmpW. Second, the recombinant protein was immunoreactive with the 5-3 pANCA monoclonal antibody. Third, OmpW was encoded by a single-copy gene and was detected exclusively in *B. caccae* (versus other species of *Bacteroides* and various gram-negative coliforms). These findings agree with the size and species distribution of the 100-kDa protein, as originally defined using Western analysis of colonic bacterial species with the 5-3 pANCA monoclonal antibody (8).

**Relationship to TonB-linked outer membrane protein family.** The OmpW protein sequence was notable for the presence of a TonB box and other clusters of homology with TonB-linked receptors. The TonB complex is an energy transduction system which powers high-affinity active transport of certain membrane receptors across the gram-negative outer membrane (7, 25). The TonB box is a conserved domain of TonB-linked outer membrane proteins, mediating their association with the TonB complex. In *Enterobacteriaceae*, this system plays an important role in iron acquisition (siderophore transport), necessary for growth in iron-limited environments, including host cell tissues (23, 25).

The OmpW protein had extensive overall homology with SusC and RagA. SusC protein is an essential receptor for uptake and utilization of certain starches and intermediate-sized maltooligosaccharides, apparently powered by a *Bacteroides* homologue of the TonB complex (28, 29). With respect to RagA, the *ragAB* locus has been validated as a virulence factor of *P. gingivalis* tissue damage and in vivo survival, and RagA and RagB are recognized targets of disease-associated periodontal antibody responses (11, 21, 22). On this basis, RagA is implicated in *P. gingivalis*-associated periodontal disease. As an analogue of both SusC and RagA, OmpW may potentially play similar roles in facilitating uptake of substrates important to commensal intestinal survival and as the target of tissue-destructive immune responses in susceptible hosts. Such a potential role makes it important to further assess the distribution of OmpW in other members of the *Bacteroides-Porphyromonas-Prevotella* group.

**Relationship of OmpW to other pANCA antigens.** A number of bacterial and mammalian proteins have been advanced as candidate pANCA antigens. The OmpW epitope recognized by the 5-3 pANCA monoclonal antibody was localized by truncation mutants. Thus, antigenicity was lost by the truncation

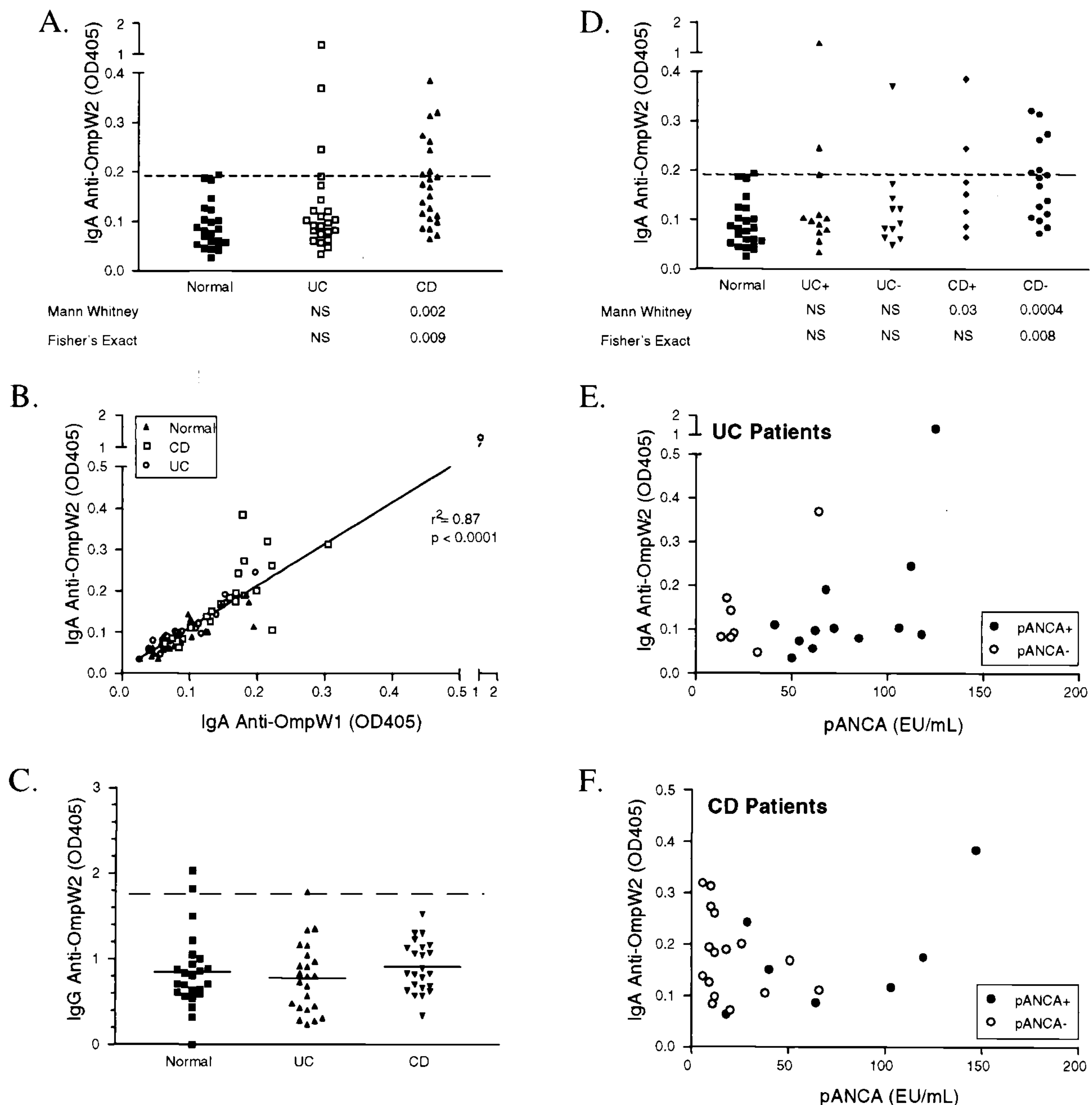


FIG. 8. Serum anti-OmpW antibody levels in normal and IBD patients. ELISA wells coated with OmpW2 (A, B, D, E, and F) or OmpW1 (B and C) were reacted with patient sera, and binding was detected by IgA- (A, B, D, E, and F) or IgG- (C) specific secondary reagents and expressed as absorbance units ($OD_{405}$). Levels of pANCA activity were expressed as enzyme units (EU) per milliliter. (A) Levels of anti-OmpW2 IgA in normal, UC, and CD patients. Quantitative values for each IBD group compared to normals were compared by a Mann-Whitney (unpaired, two-tailed). Positive sera were defined as those exceeding the mean + 2 standard deviations for the normal group (dashed line). The frequencies of positive individuals in each IBD group compared to normals were compared by a Fisher's exact test. (B) Levels of IgA antibodies to OmpW1 and OmpW2. Correlation of the values was assessed by linear regression. (C) Levels of anti-OmpW1 IgG in normal, UC, and CD patients. Dashed line is the positive cutoff value, and solid lines are the arithmetic means for each group. The standard deviations for normal, UC, and CD values are: (A) 0.044, 0.026, and 0.87; (C) 0.45, 0.40, and 0.31. (D) Levels of anti-OmpW2 IgA in patients stratified for UC-pANCA positive (+) and negative (−) immunofluorescence. The standard deviations are normal (0.044), UC+ (0.35), UC− (0.089), CD+ (0.11), and CD− (0.080). (E and F) Levels of IgA anti-OmpW2 and pANCA in UC (E) and CD (F) patients. Patients were stratified for UC-pANCA positive (solid symbols) and negative (open symbols) immunofluorescence.

from OmpW2 to OmpW3 (residues 143 to 300). This is the unique segment of OmpW bearing KKAK motifs, which were previously identified as core epitopes in three other candidate pANCA antigens (mammalian histone H1, HMG1/2, and mycobacterial HupB) (9, 16). In contrast, other candidate pANCA antigens lack the KKAK motif (*E. coli* OmpC).

The present study also demonstrates that anti-OmpW IgA does not correlate with pANCA seroreactivity in human IBD

patients. The Fab 5-3 pANCA monoclonal antibody was selected in the bacterial antigen search because it represents one of only two pANCA monoclonal antibodies reported in the literature. The other antibody, Fab 5-2, was nonreactive with OmpW, perhaps due to its sensitivity to denaturation of its cognate epitope (8). Individual monoclonal antibodies may not reflect the predominant epitopes detected by an antibody response. This issue may account for the discordance between OmpW and the predominant antigen(s) detected by pANCA serum antibodies (8). Specifically, our observations indicate that antibodies specific for the KKAK motif are a minor component of the serum pANCA repertoire. This supports the emerging view that IBD-associated pANCA immunoreactivity involves not one but several peptide motifs and conformational determinants (8).

The immunologic stimulus leading to this divergent ensemble of immunoreactivities is uncertain but is reminiscent of epitope spreading observed in other chronic antimicrobial and autoreactive immune responses (42). It may be instructive to evaluate the association of anti-OmpW activity with stratified patient subpopulations. This issue will require a much larger patient study design, to accrue sufficient numbers of subjects to analyze in a statistically meaningful way for patient subgroups with distinct clinical features (disease duration, disease activity, and treatment profile).

***Bacteroides*, OmpW, and IBD pathogenesis.** The immunologic finding in this paper is that there was an elevation of anti-OmpW IgA levels in a subset of patients with CD (compared to healthy controls and UC patients). The number of subjects in the present study was insufficient to address the potential correlation of anti-OmpW IgA with clinically or genetically defined CD patient subpopulations. A larger population study and alternate randomization strategies may also resolve potential type 1 statistical error in our conclusions regarding disease association. The immunoreactivity might be secondary to mucosal damage and increased bacterial antigen delivery across the disrupted epithelial barrier to inductive immunologic sites. In support of this idea, *Bacteroides* spp. (including *B. caccae*) are a major component of the colonic microflora and typically display a commensal, nonvirulent phenotype (19). Monoassociation studies have implicated *Bacteroides vulgatus* as a pathogenic factor in a rat transgenic model of colitis (27). CD is distinguished not only by antibody immunoreactivity to several colonic bacterial taxa (1, 3, 50), but also by a striking expansion of T cells reactive to autologous colonic bacteria, including *B. thetaiotaomicron* (14). Gut-associated T lymphocytes are remarkably anergic to colonic bacterial antigens. Similar observations have been made in mouse models of IBD, notably the capacity of such T cells to establish disease by cell transfer (10). These observations strongly implicate immune responses to one or more enteric bacterial species as an important element of mucosal damage in IBD. The present work provides specific bacterial species and protein antigens for experimental evaluation.

How commensal bacteria become harmful in a susceptible host remains to be elucidated. Overgrowth of enteric bacteria is observed in IBD patients, indicating that IBD involves disruption of the normal enteric bacterial ecosystem, caused by or resulting in host immune and inflammatory responses. It is conceivable that factors related to bacterial growth may play a role in this ecosystem disruption. Competition for scarce nutrients, notably iron, directly shapes the dynamics of bacterial populations (19). Moreover, transcriptional control of virulence traits is a common feature of signaling pathways regulated by uptake of such nutrients and quorum-sensing mechanisms (12, 24). In this context, TonB-linked proteins such as *B. caccae* OmpW may play a significant role in such processes.

Little is presently known about the prevalence and distribution of *B. caccae* in the intestine of healthy persons or IBD patients, its targeting by the disease-related immune response, and role in IBD pathogenesis evaluated through monoassociation study of bacterial virulence in IBD. The present study points to *B. caccae* and OmpW for such experimental evaluation in IBD pathogenesis.

## ACKNOWLEDGMENTS

We are especially grateful for the technical assistance of Kathleen Lechowitzc and Kevin Ward. We thank Dominica Salvatore for assistance in preparation of the manuscript.

This work was supported by NIH grants DK46763 (J.B., S.R.T.), DK43026 (S.R.T.), and AI 07126-23 (H.D., the UCLA Clinical and Fundamental Immunology Training Grant), the Crohn's and Colitis Foundation of America (W.B.), the Jonsson Comprehensive Cancer Center (J.B.), CURE Digestive Diseases Research Center (J.B.), and the Feintech Family Chair of Inflammatory Bowel Disease (S.R.T.).

## REFERENCES

1. **Auer, I. O., A. Roder, F. J. Wensinck, P. van de Merwe, and H. Schmidt.** 1983. Selected bacterial antibodies in Crohn's disease and ulcerative colitis. Scand. J. Gastroenterol. **18:**217–223.
2. **Billing, P., S. Tahir, B. Calfin, G. Gagne, L. Cobb, S. R. Targan, and A. Vidrich.** 1995. Nuclear localization of the antigen detected by ulcerative colitis-associated perinuclear antineutrophil cytoplasmic antibodies. Am. J. Pathol. **147:**979–987.
3. **Blaser, M. J., R. A. Miller, J. Lacher, and J. W. Singleton.** 1984. Patients with active Crohn's disease have elevated serum antibodies to antigens of seven enteric bacterial pathogens. Gastroenterology **87:**888–894.
4. **Blumberg, R. S., L. J. Saubermann, and W. Strober.** 1999. Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. Curr. Opin. Immunol. **11:**648–656.
5. **Boismenu, R., and Y. Chen.** 2000. Insights from mouse models of colitis. J. Leukocyte Biol. **67:**267–278.
6. **Brandwein, S. L., R. P. McCabe, Y. Cong, K. B. Waites, B. U. Ridwan, P. A. Dean, T. Ohkusa, E. H. Birkenmeier, J. P. Sundberg, and C. O. Elson.** 1997. Spontaneously colitic C3H/HeJBir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora. J. Immunol. **159:**44–52.
7. **Braun, V., S. Gaisser, C. Herrmann, K. Kampfenkel, H. Killmann, and I. Traub.** 1996. Energy-coupled transport across the outer membrane of *Escherichia coli*: ExbB binds ExbD and TonB in vitro, and leucine 132 in the periplasmic region and aspartate 25 in the transmembrane region are important for ExbD activity. J. Bacteriol. **178:**2836–2845.
8. **Cohavy, O., D. Bruckner, L. K. Gordon, R. Misra, B. Wei, M. E. Eggena, S. R. Targan, and J. Braun.** 2000. Colonic bacteria express an ulcerative colitis pANCA-related protein epitope. Infect. Immun. **68:**1542–1548.
9. **Cohavy, O., G. Harth, M. A. Horwitz, C. Landers, C. Sutton, S. R. Targan, and J. Braun.** 1999. Identification of a novel mycobacterial histone H1 homologue (HupB) as an antigenic target of pANCA monoclonal antibody and serum IgA from patients with Crohn's disease. Infect. Immun **67:**6510–6517.
10. **Cong, Y. Z., S. L. Brandwein, R. P. McCabe, A. Lazenby, E. H. Birkenmeier, J. P. Sundberg, and C. O. Elson.** 1998. CD4+ T cells reactive to enteric bacterial antigens in spontaneously colitic C3H/HeJBir mice: increased T helper cell type 1 response and ability to transfer disease. J. Exp. Med. **187:**855–864.
11. **Curtis, M. A., S. A. Hanley, and J. Aduse-Opoku.** 1999. The rag locus of Porphyromonas gingivalis: a novel pathogenicity island. J. Periodont. Res. **34:**400–405.
12. **de Kievit, T. R., and B. H. Iglewski.** 2000. Bacterial quorum sensing in pathogenic relationships. Infect. Immun. **68:**4839–4849.
13. **Dianda, L., A. M. Hanby, N. A. Wright, A. Sebesteny, A. C. Hayday, and M. J. Owen.** 1997. T cell receptor-alpha, beta-deficient mice fail to develop colitis in the absence of a microbial environment. Am. J. Pathol. **150:**91–97.
14. **Duchmann, R., E. May, M. Heike, P. Knolle, M. Neurath, and B. K. Zum.** 1999. T cell specificity and cross reactivity towards enterobacteria, Bacte-

roides, Bifidobacterium, and antigens from resident intestinal flora in humans. Gut **44:**812–818.

15. **Duerr, R. H., S. R. Targan, C. J. Landers, L. R. Sutherland, and F. Shanahan.** 1991. Anti-neutrophil cytoplasmic antibodies in ulcerative colitis: comparison with other colitides/diarrheal illnesses. Gastroenterology **100:**1590–1596.
16. **Eggena, M., O. Cohavy, M. Parseghian, B. A. Hamkalo, D. Clemens, S. R. Targan, L. K. Gordon, and J. Braun.** 2000. Identification of histone H1 as a cognate antigen of the ulcerative colitis-associated marker antibody pANCA. J. Autoimmunity **14:**83–97.
17. **Eggena, M., S. R. Targan, L. Iwanczyk, A. Vidrich, L. K. Gordon, and J. Braun.** 1996. Phage display cloning and characterization of an immunogenetic marker (perinuclear anti-neutrophil cytoplasmic antibody) in ulcerative colitis. J. Immunol **156:**4005–4011.
18. **Elson, C. O., R. B. Sartor, G. S. Tennyson, and R. H. Riddell.** 1995. Experimental models of inflammatory bowel disease. Gastroenterology **109:**1344–1367.
19. **Finegold, S. M., and V. L. Sutter.** 1978. Fecal flora in different populations, with special reference to diet. Am. J. Clin. Nutr. **1:**S116–S122.
20. **Hammer, R. E., S. D. Maika, J. A. Richardson, J. P. Tang, and J. D. Taurog.** 1990. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta2m: an animal model of HLA-B27-associated human disorders. Cell **63:**1099–1112.
21. **Hanley, S. A., J. Aduse-Opoku, and M. A. Curtis.** 1999. A 55-kilodalton immunodominant antigen of Porphyromonas gingivalis W50 has arisen via horizontal gene transfer. Infect. Immun. **67:**1157–1171.
22. **Kojima, T., K. Yano, and I. Ishikawa.** 1997. Relationship between serum antibody levels and subgingival colonization of Porphyromonas gingivalis in patients with various types of periodontitis. J. Periodontol. **68:**618–625.
23. **Larsen, R. A., P. S. Myers, J. T. Skare, C. L. Seachord, R. P. Darveau, and K. Postle.** 1996. Identification of TonB homologs in the family *Enterobacteriaceae* and evidence for conservation of TonB-dependent energy transduction complexes. J. Bacteriol. **178:**1363–1373.
24. **Litwin, C. M., and S. B. Calderwood.** 1993. Role of iron in regulation of virulence genes. Clin. Microbiol. Rev. **6:**137–149.
25. **Moeck, G. S., and J. W. Coulton.** 1998. TonB-dependent iron acquisition: mechanisms of siderophore-mediated active transport. Mol. Microbiol. **28:**675–681.
26. **Panwala, C. M., J. C. Jones, and J. L. Viney.** 1998. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. J. Immunol. **161:**5733–5744.
27. **Rath, H. C., H. H. Herfarth, J. S. Ikeda, W. B. Grenther, T. E. Jr. Hamm, E. Balish, J. D. Taurog, R. E. Hammer, K. H. Wilson, and R. B. Sartor.** 1996. Normal luminal bacteria, especially bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. J. Clin. Investig. **98:**945–953.
28. **Reeves, A. R., J. N. D'Elia, J. Frias, and A. A. Salyers.** 1996. A *Bacteroides thetaiotaomicron* outer membrane protein that is essential for utilization of maltooligosaccharides and starch. J. Bacteriol. **178:**823–830.
29. **Reeves, A. R., G. R. Wang, and A. A. Salyers.** 1997. Characterization of four outer membrane proteins that play a role in utilization of starch by *Bacteroides thetaiotaomicron*. J. Bacteriol. **179:**643–649.
30. **Reumaux, D., C. Meziere, J.-F. Colombel, P. Duthilleul, and S. Muller.** 1995. Distinct production of autoantibodies to nuclear components in ulcerative colitis and in Crohn's disease. Clin. Immunol. Immunopathol. **77:**349–357.
31. **Rolleke, S., G. Muyzer, C. Wawer, G. Wanner, and W. Lubitz.** 1996. Identification of bacteria in a biodegraded wall painting by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Appl. Environ. Microbiol. **62:**2059–2065.
32. **Rudolph, U., M. J. Finegold, S. S. Rich, G. R. Harriman, Y. Srinivasan, P. Brabet, A. Bradley, and L. Birnbaumer.** 1995. Gi2 alpha protein deficiency: a model of inflammatory bowel disease. J. Clin. Immunol. **15:**101.S-105S.
33. **Rutgeerts, P., M. Hiele, K. Geboes, M. Peeters, R. Aerts, and R. Kerremans.** 1995. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. Gastroenterology **108:**1617–1621.
34. **Sartor, R. B.** 1995. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. Gastroenterol. Clin. North Am **24:**475–507.
35. **Sartor, R. B.** 1997. Pathogenesis and immune mechanisms of chronic inflammatory bowel diseases. Am. J. Gastroenterol. **92:**5.S-11S.
36. **Satsangi, J., C. J. Landers, K. I. Welsh, K. Koss, S. R. Targan, and D. P. Jewell.** 1998. The presence of anti-neutrophil antibodies reflects clinical and genetic heterogeneity within inflammatory bowel disease. Inflamm. Bowel Dis. **4:**18–26.
37. **Saxon, A., F. Shanahan, C. Landers, T. Ganz, and S. R. Targan.** 1990. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. J. Allergy Clin. Immunol. **86:**202–210.
38. **Schwartz, R. D., and S. K. Datta.** 1989. Autoimmunity and autoimmune diseases, p. 819–866. *In* W. E. Paul (ed.), Fundamental immunology. Raven Press, New York, N.Y.
39. **Seibold, F., S. Brandwein, S. Simpson, C. Terhorst, and C. O. Elson.** 1998. pANCA represents a cross-reactivity to enteric bacterial antigens. J. Clin. Immunol. **18:**153–160.
40. **Sellon, R. K., S. Tonkonogy, M. Schultz, L. A. Dieleman, W. Grenther, E. Balish, D. M. Rennick, and R. B. Sartor.** 1998. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect. Immun. **66:**5224–5231.
41. **Shanahan, F., R. H. Duerr, J. I. Rotter, H.-Y. Yang, L. R. Sutherland, C. McElree, C. J. Landers, and S. R. Targan.** 1992. Neutrophil autoantibodies in ulcerative colitis: familial aggregation and genetic heterogeneity. Gastroenterology **103:**456–461.
42. **Shastri, N., A. Oki, A. Miller, and E. E. Sercarz.** 1985. Distinct recognition phenotypes exist for T cell clones specific for small peptide regions of proteins. J. Exp. Med. **162:**332–345.
43. **Smith, C. J.** 1985. Development and use of cloning systems for *Bacteroides fragilis*: cloning of a plasmid-encoded clindamycin resistance determinant. J. Bacteriol. **164:**294–301.
44. **Struyve, M., M. Moons, and J. Tommassen.** 1991. Carboxy-terminal phenylalanine is essential for the correct assembly of a bacterial outer membrane protein. J. Mol. Biol. **218:**141–148.
45. **Tan, E. M.** 1991. Autoantibodies in pathology and cell biology. Cell **67:**841–842.
46. **Tommassen, J., M. Struyve, and H. de Cock.** 1992. Export and assembly of bacterial outer membrane proteins. Antonie Van Leeuwenhoek **61:**81–85.
47. **Toyoda, H., S.-J. Wang, H.-Y. Yang, A. Redford, D. Magalong, D. Tyan, C. McElree, S. Pressman, F. Shanahan, S. R. Targan, and J. I. Rotter.** 1993. Distinct associations of HLA class II genes with inflammatory bowel disease. Gastroenterology **104:**741–748.
48. **van J. P. de Merwe, A. M. Schroder, F. Wensinck, and M. P. Hazenberg.** 1988. The obligate anaerobic faecal flora of patients with Crohn's disease and their first-degree relatives. Scandinavian J. Gastroenterology **23:**1125–1131.
49. **Vidrich, A., J. Lee, E. James, L. Cobb, and S. R. Targan.** 1995. Segregation of pANCA antigenic recognition by DNase treatment of neutrophils: ulcerative colitis, type 1 autoimmune hepatitis, and primary sclerosing cholangitis. J. Clin. Immunol. **15:**293–299.
50. **Wayne, L. G., D. Hollander, B. Anderson, H. A. Sramek, C. M. Vadheim, and J. I. Rotter.** 1992. Immunoglobulin A (IgA) and IgG serum antibodies to mycobacterial antigens in Crohn's disease patients and their relatives. J. Clin. Microbiol. **30:**2013–2018.
51. **Yang, H.-Y., J. I. Rotter, H. Toyoda, C. Landers, D. Tyan, C. K. McElree, and S. R. Targan.** 1993. Ulcerative colitis: a genetically heterogeneous disorder defined by genetic (HLA class II) and subclinical (antineutrophil cytoplasmic antibodies) markers. J. Clin. Investig. **92:**1080–1084.

*Editor:* J. D. Clements