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CS22, a Novel Human Enterotoxigenic *Escherichia coli* Adhesin, Is Related to CS15

MARIANA PICHEL, NORMA BINSZTEIN, AND GLORIA VIBOUD*

Departamento de Bacteriología, Instituto Nacional de Enfermedades Infecciosas-ANLIS "Carlos G. Malbrán," 1281 Capital Federal, Argentina

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Enterotoxigenic *Escherichia coli* (ETEC) expresses a broad spectrum of O:H antigens. Serogroup O20 is one of the most prevalent among the ETEC strains lacking any of the defined colonization factors (CFs), in Argentina. An O20:H− strain, ARG-3, adhered to Caco-2 cells and exhibited a thermoregulated 15.7-kDa protein band upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). An antiserum against this protein inhibited ARG-3 adhesion to Caco-2 cells and bound to very thin fibrilla-like structures on the bacterial surface. A 15.7-kDa protein-defective mutant failed to adhere to Caco-2 cells and lacked immunogold-labeled surface structures. The N-terminal amino acid sequence of the structural subunit showed 95% homology to that of CS15 of ETEC (former antigen 8786) and 65% homology with fimbria SEF14 of *Salmonella enterica* serovar Enteritidis. Nevertheless, the molecular size of ARG-3 adhesin was different from that of CS15, as revealed by SDS-PAGE and mass spectrometry. Both proteins are immunologically related, yet not identical, since an antiserum against the 15.7-kDa protein reacted solely with ARG-3 after absorption with bacteria bearing CS15. Moreover, only under low stringency conditions could DNA from strain ARG-3 be amplified by PCR using primers derived from the *nfaA* sequence of CS15. Thus, from the DNA sequence obtained from the ARG-3 PCR product, it could be deduced that the subunit protein differed in 30 residues from that of CS15. ARG-3 adhesin was found in 60% of the O20:H- CF-negative ETEC strains from Argentina; however, it appeared restricted to this serotype. We propose the designation CS22 for the herein identified nonfimbrial adhesin of human ETEC.

Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause of diarrhea in children in developing countries and in travelers to those areas (5). ETEC produces heat-labile enterotoxins, heat-stable enterotoxins, or both, which induce a net secretion of electrolytes and water to the gut lumen. The ability to adhere to enterocytes and to colonize the small intestine is essential for ETEC pathogenicity and is conferred by colonization factors (CFs) (11). The different CFs may be fimbrial, nonfimbrial or fibrillar structures, and they have been grouped in four big families according to the homology in their N-terminal amino acid sequence (11). Recently, Gaastra and Svennerholm (11) revised the nomenclature of these antigens, based on the designation proposed by M. M. McConnell. Thus, the CFs described so far include: CFA/I, CS1 to CS7, CS8 (originally CFA/III), CS10 (antigen 2230), CS11 (PCFO148), CS12 (PCFO159), CS13 (PCFO9), CS14 (PCFO166), CS15 (antigen 8786), CS17, CS18 (PCFO20), CS19, CS20, and CS21 (longus). Each CF has a unique subunit molecular mass as determined by studies of spray mass spectrometry (F. J. Cassels et al., unpublished data). Most of them are encoded by genes located on high-molecular-weight plasmids (10, 18, 20), and the expression of these genes is thermoregulated by the histone-like protein H-NS (7). The ability of several of these CFs to promote colonization and induce immune response has been shown in experimental animals (19) and human volunteers (9).

Surveys of ETEC isolates have shown that most CFs are associated with a limited number of O:H serotypes (4, 21, 25). Epidemiological studies carried out in Argentina (4, 21, 22) revealed that there is a high proportion (35 to 40%) of ETEC strains isolated from children with diarrhea that do not express any of the defined CFs. Serogroup O20 was one of the most prevalent among these CF-negative ETEC isolates. These findings, along with the recent identification of CS18 in an O20:H− Argentinean strain, led us to search for other adhesins on the ETEC isolates belonging to this O group. Since antibacterial immunity induced by ETEC is to a large extent CF specific (1, 6), it is essential to study the distribution of the known CFs in different geographical areas, as well as the emergence of new adhesins, in order to design effective ETEC vaccines. By assessing the ability of CF-negative strains to adhere to Caco-2 cells, we identified a previously undescribed colonization factor on the O20:H−, ST ETEC strain ARG-3, isolated from a child with diarrhea in Argentina.

MATERIALS AND METHODS

Bacterial strains and culture conditions. All ETEC strains used in the study were kept at −70°C in Trypticase soy broth supplemented with 15% glycerol and were grown in CF antigen (CFA) agar containing 1.5 g of Bacto Bile Salts no. 3 (Difco, Detroit, Mich.) per liter (CFA-BS agar) (14), or in Trypticase soy agar (TSA) at 37°C overnight.

Preparation of bacterial heat extracts. Bacterial suspensions of overnight cultures of the ETEC strains were heated at 60°C for 30 min. After centrifugation for 10 min at 2,000 × *g*, supernatants were separated and kept at −20°C until use.

Dot blot. Bacterial heat extracts or whole bacteria were spotted on nitrocellulose membranes and reacted with specific monoclonal antibodies raised against CFA/I, CS1 to CS8, CS12, and CS14, provided by Ann-Mari Svennerholm, or with polyclonal antiserum against CS15, provided by Arlette Darfeuille-Michaud, or CS18 (24). The antibody-antigen complexes were detected with immunoglobulin G (IgG) antibody labeled with horseradish peroxidase and developed with hydrogen peroxide substrate and 4-chloro-naphthol chromogen, as described elsewhere (21).

SDS-PAGE and immunoblotting. Crude protein extracts and purified fimbrial proteins were electrophoresed through sodium dodecyl sulfate–20% polyacrylamide gel electrophoresis (SDS–20% PAGE) gels according to the method described by Laemmli (12). The gels were either stained with Coomassie brilliant

* Corresponding author. Present address: Department of Molecular Genetics and Microbiology, School of Medicine, State University of New York at Stony Brook, Stony Brook, NY 11794-5222. Phone: (631) 632-8783. Fax: (631) 632-9797. E-mail: gviboud@ms.cc.sunysb.edu.

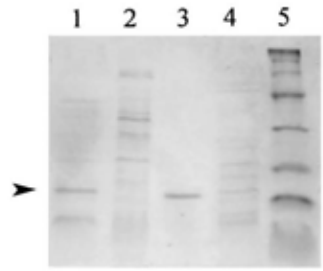

FIG. 1. SDS-PAGE analysis of purified adhesin subunit and heat extracts from ETEC strains grown on CFA-BS agar. Lanes: 1, heat extract from strain ARG-3 grown at 37°C; 2, heat extract from strain ARG-3 grown at 22°C; 3, purified adhesin from strain ARG-3; 4, heat extract from mutant strain ARG-3/247; 5, molecular mass marker (broad-range standard, Bio-Rad): aprotinin (6.5 kDa), lysozyme (14.4 kDa), soybean trypsin inhibitor (21.5 kDa), carbonic anhidrase (31 kDa), ovalbumin (45 kDa), bovine serum albumin (66.2 kDa), phosphorylase *b* (97.4 kDa), β-galactosidase (116.25 kDa), and myosin (200 kDa).

blue R250, or electroblotted to nitrocellulose membranes using the Mini Trans-Blot electrophoretic transfer cell (Bio-Rad Laboratories, Hercules, Calif.). The membranes were incubated overnight at room temperature with antifimbrial rabbit serum and developed as described above.

Serotyping. ETEC strain ARG-3 was kindly serotyped by Richard Wilson at the Department of Veterinary Science, Pennsylvania State University. The strains from our ETEC culture collection were serotyped by Ida Ørskov, International *Escherichia* and *Klebsiella* Center, Statens Seruminstitut, Copenhagen, Denmark.

Purification of fimbrial proteins. Whole-cell suspension of strain ARG-3 was prepared by growing bacteria at 37°C overnight on CFA agar. Bacteria were harvested with phosphate-buffered saline (PBS), and the suspension was homogenized using an Omnimixer blender for 5 min at maximal speed in an ice bath. The homogenate was centrifuged at 12,000 × *g* for 20 min at 4°C. Purified protein was obtained after serial precipitations with ammonium sulfate (20, 40, and 60%) as previously described (8).

Preparation of antisera and Fab fragments. Rabbit immune antiserum was obtained following four subcutaneous injections with 100 μg of purified protein at 2-week intervals, as previously described (24). Specific anti-ARG-3 adhesin serum (C238) was obtained by absorption with ARG-3 bacteria grown at 22°C. The IgG fraction of this serum was purified by affinity chromatography in a protein A column (Bio-Rad Laboratories). After digestion with papain, the Fab fragments were purified by absorbing the intact IgG and the Fc fraction in a protein A column.

Adhesion to cultured human colon carcinoma cell line Caco-2. Caco-2 adhesion assay was performed as previously described, with some modifications (23). After incubating the cells with the bacterial suspensions for 3 h, the samples were washed five times with PBS and treated with 200 μl of Triton X-100 (0.5% in PBS) for 10 min at 37°C, and serial dilutions in PBS were plated by duplicate on TSA to determine the number of adherent bacteria/milliliter. The mean number of bacteria/cell was calculated by dividing the number of bacteria/milliliter by the number of Caco-2 cells/milliliter. Alternatively, after washing with PBS, cells were fixed with methanol and treated with 10% Giemsa stain for light microscopy examination.

Adhesion inhibition tests. Adhesion inhibition by Fab fragments of extensively absorbed C238 antiserum was tested by mixing equal volumes of Fab fragment preparation and a suspension of 8×10^8 bacteria/ml. The mixture was incubated at room temperature for 20 min and added to the Caco-2 cells. The adhesion assay was performed as described above. CS8, CS6-positive strain 34420A, and CS12-positive strain $350C_1A$, mixed with the same preparation of Fab fraction, were used as unrelated controls.

N-terminal amino acid sequence determination. Crude protein extracts from ARG-3 strain were separated by SDS-PAGE as described above, and electroblotted to a ProBlott (PEByosystems, Foster City, Calif.) immobilization membrane. After staining with 1% Ponceau S strain, the 15.7-kDa adhesin subunit band was excised and sent for N-terminal amino acid sequence analysis to the Protein Structure Core Facility, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center.

Electron microscopy. Strain ARG-3 was grown overnight at 37 and at 22°C on CFA-BS agar, suspended in distilled water, and applied on Formvar-coated nickel grids (200 mesh). The grids were either negatively stained with 1% ammonium molybdate or developed by immunogold labeling, using absorbed C238 antiserum and gold-labeled protein A (Auro Probe EM; Amersham International, Little Chalfont, United Kingdom) as previously described (24). The grids were then examined in an electron microscope (model EM109, Zeiss, Oberkochen, Germany).

Mass spectrometry. The mass of the adhesin protein subunit was determined by electrospray mass spectrometry (F. Cassels, L. Pannell, J. Barringer, J. Anderson, M. Lisher, S. F. Khalil, and S. J. Savarino, Abstr. 97th Gen. Meet. Am. Soc. Microbiol. 1997, abstr. D-95, 1997) at the laboratory of Frederick Cassels, Walter Reed Army Institute of Research, Washington, D.C.

Mutagenesis. Mutagenesis was performed on strain ARG-3 by treatment with the alkylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) (15). Briefly, late log-phase culture of ARG-3 was exposed to NTG for 10 min at 37°C, and after washing the cells to remove the mutagen, the bacteria were suspended in PBS and plated onto CFA-BS agar. Mutants that did not react with the absorbed antiadhesin serum in dot blots were selected for further analyses.

PCR. Two pairs of primers were designed based on the sequence of the *nfaA* gene of CS15 antigen (2), to amplify two fragments: one of 628 bp, encompassing the whole structural gene and an inner fragment of 156 bp (primers 1, ATAAAAAATAAAGCACGCAG, and 2, CAACGTCAGCATCTTACAGT; and primers 3, TAAATGGGATAGTAATGAGG, and 4, CCTCTGTATCTGGTTCTTCA, respectively). An aliquot of 50 μl of the reaction mixture contained 100 nmol of $MgCl_2$, 0.4 U of *Taq* polymerase, 5 μl of 10× buffer, 1.25 nmol of each deoxynucleoside triphosphate, 25 pmol of each primer, and 2 μl of DNA preparation. The amplification reaction consisted of 10 min at 94°C; 30 cycles of 94°C

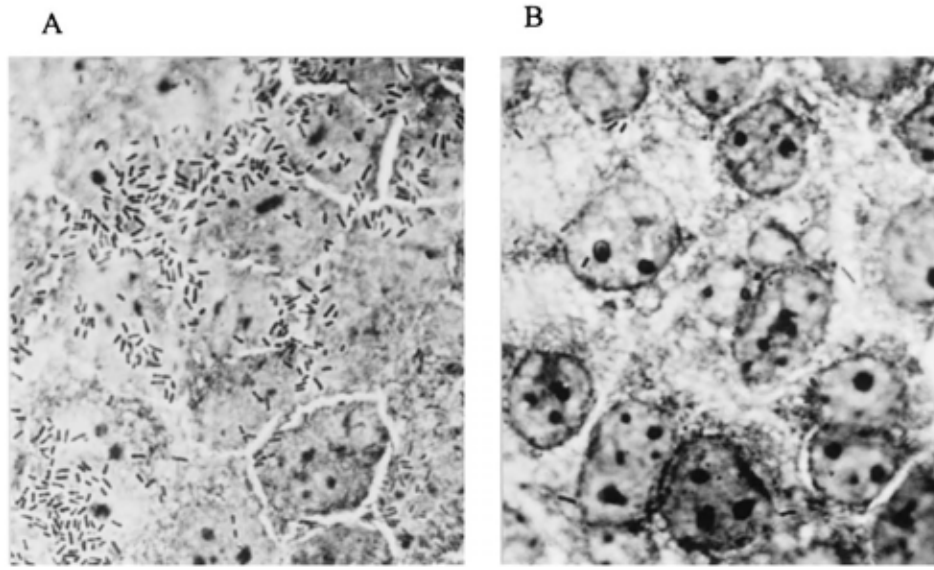

FIG. 2. Micrographs showing adhesion and adhesion inhibition of ETEC strain ARG-3 to Caco-2 cells. (A) Strain ARG-3; (B) adhesion inhibition of strain ARG-3 to Caco-2 cells using Fab fragments of monospecific C238 antiserum raised against purified adhesin.

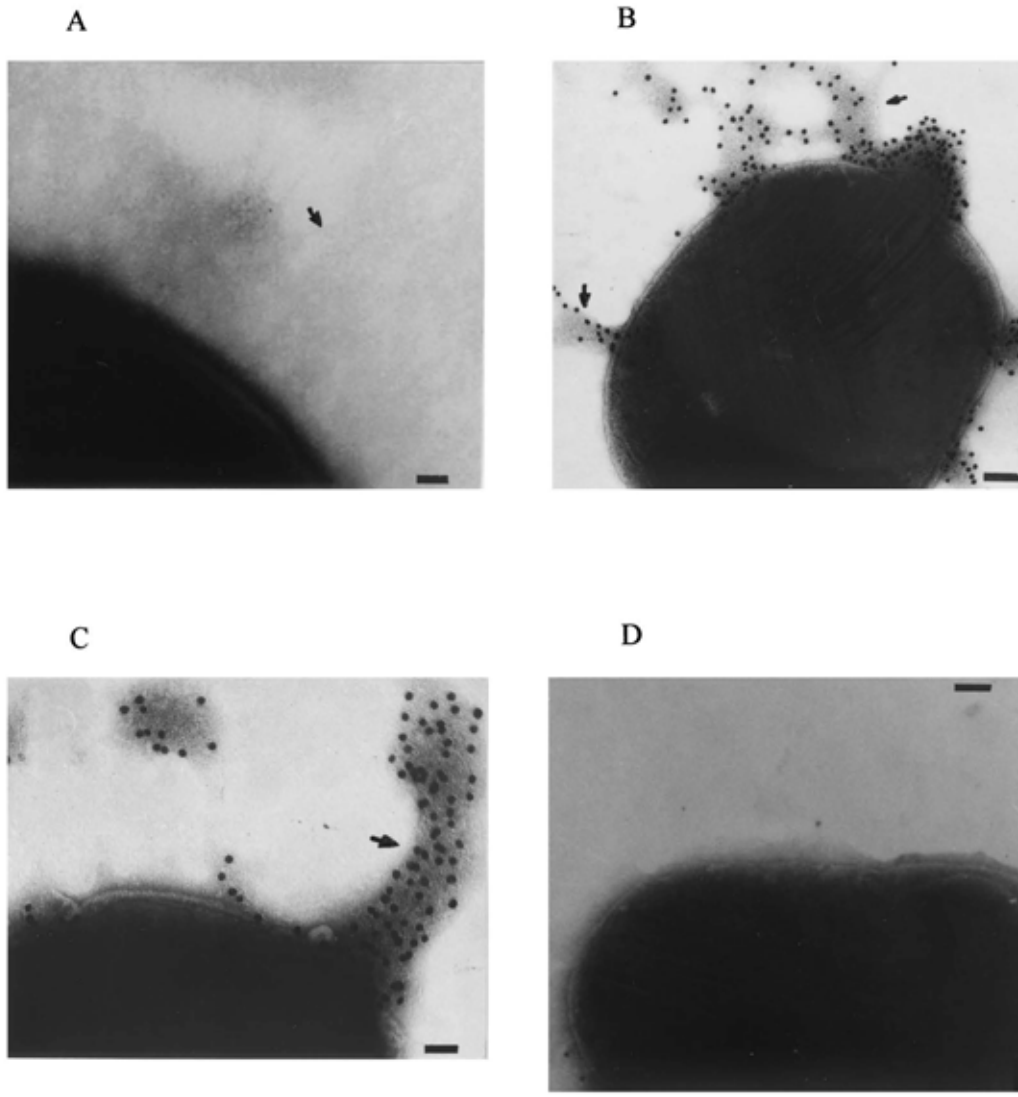

FIG. 3. (A) Electron micrographs showing strain ARG-3 grown at 37°C negatively stained with 1% ammonium molybdate (bar, 20 nm). (B) Strain ARG-3 grown at 37°C after immunogold labeling with specific C238 antiserum (bar, 100 nm). (C) Same preparation as in panel B, but at a higher magnification (bar, 50 nm). (D) Strain ARG-3 grown at 22°C, gold immunolabeled with C238 antiserum (bar, 100 nm).

for 1 min, 57.1°C (low stringency) or 64.9°C (high stringency) for 1 min, and 72°C for 1 min; and a final extension of 10 min at 72°C. The DNA used as template consisted of a bacterial suspension in water (optical density at 620 nm, approximately 1), boiled for 10 min. Equal amounts of the PCR products (10 μl) were loaded in 1.2% agarose gels.

Nucleotide sequence accession number. A 628-bp PCR product from strain ARG-3 was sequenced at the Centro de Investigación en Ciencias Agropecuarias, INTA, Buenos Aires, Argentina. Nucleotide or protein database searches were performed using Blast Network Service. The sequence obtained was submitted to GenBank under accession no. AF145205.

RESULTS

Strain ARG-3 produces a thermoregulable surface protein. Among a group of CF-negative O20 ETEC strains isolated from children with diarrhea in different areas of Argentina, a cluster of genetically related isolates was identified in a previous study (M. Pichel, N. Binsztein, and G. I. Viboud, unpublished data). These strains were shown to be related, yet not identical, by random amplified polymorphic DNA and pulsed-field gel electrophoresis (PFGE) analyses. An ST-O20:H− strain, ARG-3, representing this group, was chosen for further characterization. Heat extracts from this isolate exhibited a strong band of 15.7 kDa upon SDS-PAGE when the bacteria were grown at 37°C (either on CFA with or without BS or on TSA), that was absent when grown at 22°C (Fig. 1). Serial ammonium sulfate precipitation and centrifugation of crude bacterial heat extracts allowed the purification of the ARG-3 temperature-dependent protein (Fig. 1). This protein was used as an immunogen to produce a monospecific polyclonal antiserum (C238), which was subsequently absorbed with strain ARG-3 grown at room temperature.

The surface protein is an adhesin. ETEC ARG-3 adhered to Caco-2 cells with 7.2 ± 0.39 bacteria/cell (mean ± standard deviation) (Fig. 2A). IgG purified from antiserum C238 was digested with papain, and the resulting Fab fragments were used to determine inhibition of ARG-3 adhesion. Thus, incubation of the C238 Fab fragments with ARG-3 strain dramatically decreased the adhesion index to 0.75 ± 0.25 bacteria/cell ($P = 0.0026$) (Fig. 2B). In contrast, no significant inhibition was observed when strain 34420A, expressing the unrelated adhesins CS8 and CS6, or strain $350C_1A$, expressing CS12, was

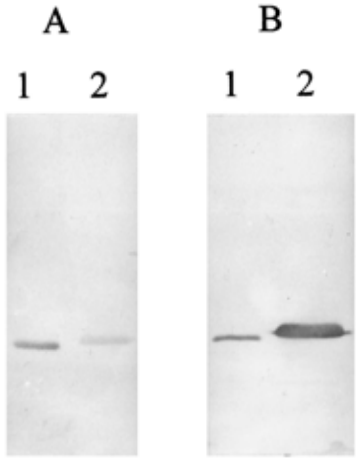

FIG. 4. Western blot analyses of heat extracts from ETEC strains ARG-3 and 8786 grown at 37°C using absorbed C238 antiserum raised against purified adhesin from ARG-3 (A) or specific anti-CS15 serum (B). Lanes: 1, ARG-3; 2, 8786.

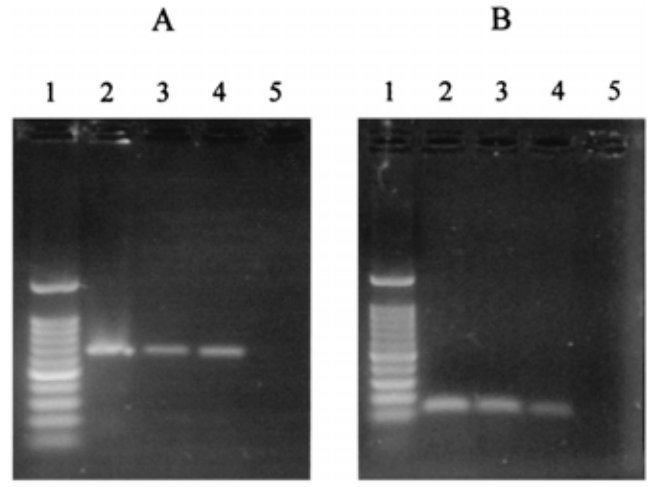

FIG. 5. Agarose gel electrophoresis patterns of PCR amplicons obtained with primers 1 and 2 (A) and primers 3 and 4 (B) derived from the sequence of gene *nfaA*, which codes for the structural subunit of CS15. Lanes: 1, molecular mass marker (100-bp ladder; Bio-Rad); 2 and 4, strain 8786; 3 and 5 strain ARG-3. Amplifications under low-stringency conditions are shown in lanes 2 and 3 and those under high-stringency conditions are shown in lanes 4 and 5.

preincubated with the same preparation (14.5 ± 2.1 and 13.0 ± 1.4 bacteria/cell [$P = 0.49$] and 19.0 ± 1.4 and 17.8 ± 1.8 bacteria/cell [$P = 0.54$], respectively).

Electron microscopy examination revealed the presence of a mesh of very thin, flexible structures on the surface of strain ARG-3 when bacteria were grown at 37°C (Fig. 3A). These organelles surrounded the bacteria, and their small diameter made them very difficult to visualize by negative staining. However, immunogold labeling using C238 serum, showed that gold particles bound to this mesh of fibrilla-like structures present on the surface of ARG-3 grown at 37°C (Fig. 3B and 3C). These organelles appeared organized into bundles in the presence of the antibody and seemed to be easily detached from the bacteria, since gold particles were also seen apart from the cells. On the other hand, when bacteria were cultured at 22°C, no labeling occurred (Fig. 3D).

The new adhesive factor is related to CS15. Strain ARG-3 tested negative in different immunoassays for CFA/I, CS1 to CS8, CS12, CS14, CS17, and CS19. The sera against two related ETEC fimbriae, i.e., CS18 and CS20, reacted weakly with ARG-3 adhesin; however, they differ from ARG-3 adhesin in respect of morphology and molecular size. The N-terminal residues of the 15.7-kDa protein band were sequenced. The resulting sequence was different from those of the most prevalent ETEC CFs. However, it was found to be homologous to that of CS15 adhesin of ETEC (3), with 85% (17 of 20) identical and 95% similar residues. In addition, the sequence obtained was also similar to that of SEF14 fimbriae of *Salmonella enterica* serovar Enteritidis (16), with 55% (11 of 20) identical and 65% conserved residues. Direct comparison between strains ARG-3 and 8786 in SDS-PAGE showed differences in migration of the adhesive subunits; i.e., 15.7 kDa versus 16.3 kDa (Fig. 4). Anti-CS15 serum, donated by A. Darfeuille, reacted with the 15.7-kDa band, albeit more weakly than with the homologous antigen (Fig. 4A). When C238 serum was used as the antibody, an analogous cross-reaction was observed with CS15 antigen (Fig. 4B). Thus, this indicates that the two proteins are immunologically related. However, after absorption with strain 8786 bearing CS15, C238 antiserum reacted solely with strain ARG-3, showing that the proteins are not identical (data not shown).

The mass of the ARG-3 protein subunit was experimentally determined by electrospray mass spectrometry at the laboratory of Fred Cassels and found to be 15,024.15 Da. In contrast, the CS15 mass calculated from the amino acids deduced from the DNA sequence (2) and determined by mass spectrometry (F. Cassel, personal communication) was considerably higher (15,348 Da). To further evaluate the differences between CS15 and ARG-3 adhesive factor, a PCR assay was designed, using two pairs of primers based on the sequence of the *nfaA* gene of the CS15 protein subunit. Under high-stringency conditions, both the 628 bp encompassing the whole structural gene and 156-bp inner fragment were amplified from strain 8786 only. However, at lower annealing temperatures both fragments were amplified from 8786 as well as from ARG-3 (Fig. 5), suggesting that there are differences in the sequence corresponding to at least one of the primers used in each reaction.

Subsequently, the nucleotide sequence of the 628-bp PCR product was determined and found to contain an open reading frame of 498 nucleotides coding for a polypeptide of 166 amino acids. Homology searches using BLAST revealed that the deduced amino acid sequence of this gene was highly homologous to those of CS15 and SEF14 fimbriae of *S. enterica* serovar Enteritidis, sharing 81 and 46% of the residues respectively (Fig. 6). The structural subunit gene of ARG-3 adhesin was designated *cseA*, and the sequence was submitted to GenBank under accession no. AF145205.

A mutant strain lacks adhesive capacity. After mutagenesis with NTG, 500 colonies were screened by dot blot assay with absorbed C238 antiserum. A stable clone, which did not react with the highly absorbed antiserum, was obtained. Heat extract from mutant ARG-3/247 lacked the 15.7-kDa protein subunit (Fig. 1A). Moreover, when this strain was tested in the Caco-2 adhesion assay, the number of bound bacteria was almost 10 times less than the number observed for the wild-type strain ARG-3 (0.73 ± 0.30 bacteria/cell versus 7.25 ± 0.39). In addition, strain ARG-3/247 did not react with serum C238 in immunoelectron microscopy (data not shown).

Prevalence of the new CF. A total of 345 ETEC isolates from children with diarrhea and healthy controls in Argentina (4, 21, 22), including strains of various serotypes (O78:H12, O78:H−, O153:H45, O6:H16, O20:H−, O20:H34, O29:H10, and O114:

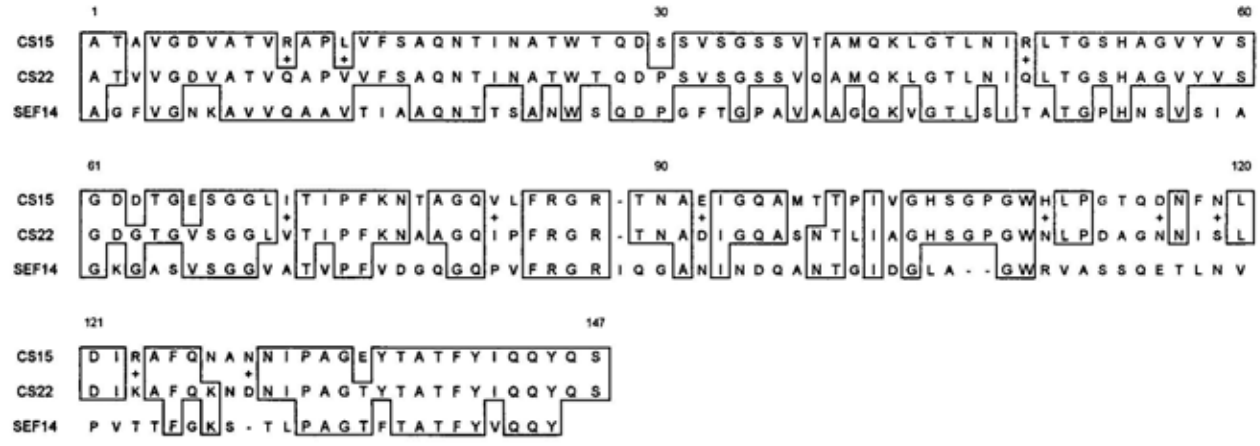

FIG. 6. Comparison of the deduced amino acid sequence of CS22 structural subunit with those of CS15 and *S. enterica* serovar Enteritidis fimbria SEF14. Identical amino acids are boxed, and well-conserved residues are marked with a +.

H21), were screened for the expression of the new adhesive factor by dot blot assay, using specific C238 antiserum absorbed with a CS15-positive strain, and further confirmed by Western blotting. ARG-3 adhesin was found restricted to serotype O20:H−, accounting for about 60% (7 of 12) of the CF-negative strains of this serotype. These isolates appeared to be genetically related, albeit not identical, as determined by random amplified polymorphic DNA and PFGE analyses (Pichel et al., unpublished data). Moreover, they had no epidemiological links; thus, they proceeded from four different regions of the country and had been isolated over a period of 5 years. None of the tested strains was positive for CS15.

DISCUSSION

Many epidemiological studies have been carried out to determine the distribution of ETEC CFs in different geographical areas (25). Surveys in Argentina, as well as in other locations, have shown that a high proportion of ETEC strains does not possess any of the hitherto-defined CFs (4, 21, 22). In the present report, we describe the identification of a new CF of human ETEC in strain ARG-3 that belongs to serogroup O20, one of the most prevalent among the CF-negative ETEC strains in Argentina (4, 21, 22). In line with the recently proposed nomenclature (11) we name this new adhesin CS22.

Several findings support the idea that the 15.7-kDa protein present in the heat extracts of strain ARG-3 is the subunit of the CS22 adhesin observed on the surface of the bacteria, i.e., that the expression of this protein is temperature dependent, as is the case for all the CFs of ETEC, and the antiserum raised against this protein bound to the very thin organelles on the surface of the bacteria, as shown by immunogold electron microscopy. Moreover, a nonadhesive mutant strain defective in the 15.7-kDa protein did not produce fibrilla-like surface structures.

The Caco-2 cell line has proven to be an excellent model to study the adhesion of human enteropathogens to the intestinal epithelium (23). The cells, derived from a human colonic carcinoma, differentiate into an epithelial cell layer, which resembles the small intestinal epithelium. In the present study, the Fab fragments of the antiserum raised against the purified structural subunit of CS22 and exhaustively absorbed specifically inhibited the binding of ARG-3 bacteria to Caco-2 cells, from which we conclude that CS22 is responsible for the adhesion to these cells. The inhibition is specific, since the Fab fraction of C238 antiserum did not significantly affect the adhesion of bacteria bearing unrelated adhesins CS8 or CS12. Furthermore, a mutant strain derived from ARG-3 that did not express the new adhesin, as seen by electron microscopy, SDS-PAGE, and immunoblot analyses, showed a significantly reduced adhesion index to Caco-2 cells, confirming the role of CS22 in conferring the ability to adhere to the intestinal epithelium.

The specific antiserum raised against CS22 showed cross-reaction with the protein subunit of antigen CS15, and an analogous cross-reaction was observed between the specific anti-CS15 serum and the structural subunit of CS22. This immunological relatedness is in line with the high homology observed between the deduced amino acid sequences of these CFs (81% identity, 87% similarity). However, after absorption with bacteria bearing CS15, the anti-CS22 serum reacted only with ARG-3, suggesting that the adhesins have both common as well as unique epitopes. Furthermore, the mass of the CS22 protein subunit calculated by electrospray mass spectrometry differs from that of CS15. This technique measures the mass of a protein to an accuracy of about 1 part in 10,000 and, therefore, it has been proposed as a method of identification of ETEC CFs (Cassels et al., Abstr. 97th Gen. Meet. Am. Soc. Microbiol.). The difference in size of the two adhesins was also observed by SDS-PAGE. The subunit proteins of both CS15 and CS22 appeared to have higher molecular sizes by SDS-PAGE than by mass spectrometry, probably due to the effect of the SDS molecules bound to the proteins.

The PCR assay using primers based on the sequence of the *nfaA* gene shows that, although closely related, the nucleotide sequences of the structural subunit genes of CS22 and CS15 are not identical, since only under low-stringency conditions was the reaction positive for ARG-3. In fact, the nucleotide sequence of the CS22 structural gene (*cseA*) differed in 41 of 498 nucleotides from that of CS15.

CS15 has been described as a nonfimbrial adhesin. However, as mentioned by the authors (3), the disposition of the gold particles in the immunoelectron microscopy examination of strain 8786 strongly suggests that the antiserum was bound to fibrilla-like structures. Furthermore, this observation was confirmed in our laboratory (data not shown), and the arrangement observed closely resembles that of CS22 and other members of the nonfimbrial adhesin group. Nevertheless, no significant homology was found between CS22 and the amino

acid sequences of adhesins nonfimbrial F1845, Dr, AFA I, and AFA III.

The deduced amino acid sequence of the CS22 subunit was, however, very similar to that of *S. enterica* serovar Enteritidis SEF14 fimbria. Interestingly, a sequence showing extensive homology to the insertion element IS*3* of *E. coli* was found upstream of the gene coding for SEF14 (16), suggesting that this virulence factor could have been transferred horizontally between the two species. It is worth noting, however, that CS22 and SEF14 have distinct morphologies, probably due to differences in the tertiary structure of their subunits. It would be interesting to evaluate if the homology in the structural gene extends to other genes of the fimbrial operon, such as those involved in the biogenesis of the organelles.

CS22 was found in almost 60% of the CF-negative O20:H− Argentinean strains tested. Although restricted to this phenotype, the new adhesin was identified in strains isolated from different locations that were shown to be genotypically related, but not identical, as determined by PFGE. This finding is in agreement with the observation of Wolf (25) that certain combinations of phenotypic traits (serotype, toxin profile, and CF) are more prevalent among ETEC and can be widespread over distant places. Examples of this are the LT/ST-O6:H16 CFA/II (17), ST-O153:H45 CFA/I (A. B. F. Pacheco, L. C. S. Ferreira, M. G. Pichel, D. F. Almeida, N. Binsztein, and G. I. Viboud, unpublished data), and LT-O114:H21 CS17 strains. It would therefore be important to evaluate the presence of CS22 in strains from other areas, especially in those in which O20 serogroup is prevalent.

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