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Monoclonal Antibody Detection of IncF Group Plasmid-Encoded TraT Protein in Clinical Isolates of *Escherichia coli*

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The TraT protein specified by IncF group plasmids mediates surface exclusion and bacterial resistance to the lethal activities of serum. In this study, an anti-TraT protein monoclonal antibody was generated which failed to react with TraT$^+$ bacteria but which efficiently detected solubilized TraT protein in Western blots and in an enzyme-linked immunosorbent assay. Use of this antibody to screen clinical and nonclinical isolates of *Escherichia coli* for the production of TraT protein revealed its presence in a modest proportion (38%) of normal fecal strains, a significantly higher proportion of clinical strains (51 to 73%), and an even higher proportion (78 to 88%) of clinical strains concomitantly producing the K1 capsule, an important virulence factor of *E. coli*.

The ability of invasive gram-negative bacterial pathogens to cause generalized infections undoubtedly results from their elaboration of a constellation of products either that protect the bacterium from the battery of host defenses it encounters or that damage the host (see, for example, reference 17). Crucial first-line host defenses against invasive pathogens are the bactericidal activities of blood, especially complement, and phagocytes; invasive pathogens must either avoid or attack these defenses or resist their action. One type of pathogenesis factor clearly involved in bacterial resistance to complement and phagocytosis is capsules, but other components, such as lipopolysaccharides and outer membrane proteins, are also able to fulfill one or both of these roles (see, for example, reference 14).

One outer membrane protein exhibiting these properties that has been studied in some detail is the TraT protein (traTp) of *Escherichia coli*. This protein, which is encoded by IncF group conjugative plasmids, mediates both resistance to the complement system (18) and plasmid surface exclusion (1). Experiments with laboratory strains of *E. coli*, isogenic except for the *traT* character, have shown that traTp is able to increase bacterial resistance not only to the lethal activities of serum but also to phagocytosis by macrophages, and bacterial virulence, as measured by the 50% lethal dose for a mouse infection model (Agüero, DeLuca, Timmis, and Cabello, submitted for publication). Although these studies have demonstrated the pathogenic potential of the traTp under experimental conditions, they did not reveal its prevalence among pathogenic bacteria or the extent of its contribution to the virulence of specific invasive coliforms. To address the first of these questions, we have produced a monoclonal antibody to screen for the presence of traTp in clinical isolates of *E. coli*. These experiments have revealed a greater prevalence of traTp in clinical isolates of *E. coli* than in isolates from healthy individuals and a particularly high incidence in K1 capsule-producing bacteria.

MATERIALS AND METHODS

Bacterial plasmids and strains. Plasmid pKT107 (18) is a hybrid plasmid composed of the cloning vector pACYC184 (7) linked to *Eco*RI fragment E-7 of plasmid R6-5, which contains the *traT* gene of this plasmid. pKT146 is a hydroxylamine-generated mutant derivative of pKT107 that overproduces traTp (16). Two derivatives of the laboratory strain of *E. coli* K-12 C600 Rif^r (4), containing either pKT107 or pACYC184, served as reference strains for the presence or absence of traTp. The former, and occasionally C600 Rif^r(pKT146), served as sources of traTp for its purification. Bacteria were routinely cultured in tryptic soy broth (TSB) containing, in the case of bacteria carrying plasmids pACYC184, pKT107, or pKT146, tetracycline at a concentration of 10 μg/ml. Strains from patients of the Mainz University hospitals and several other hospitals in the Rhine-Main area, who were suffering from gram-negative septicemia, upper-urinary-tract infections (UTI), or diarrhea in early childhood, were isolated in the diagnostic laboratories of the Institute of Medical Microbiology, University of Mainz. *E. coli* strains from feces of healthy donors were isolated from persons applying for positions at the University hospitals. *E. coli* strains from children with enteritis were typed with antisera against O and K antigens (Behring-Werke, Marburg, Federal Republic of Germany), and strains with the following O and K patterns were selected: O26:K60, O55:K59, O78:K80, O111:K58, O119:K69, O125:K70, O127:K63, and O128:K67. All strains were isolated by routine culture techniques and confirmed as *E. coli* by their fermentation and enzyme patterns with the commercial API 20E system.

K1 typing. *E. coli* strains producing the capsule antigen K1 were identified by halo formation on nutrient agar plates (20) containing an anti-meningococcus B polysaccharide antiserum, kindly provided by J. B. Robbins, Bethesda, Md. K1 capsule-specific bacteriophages (11) kindly provided by B. Rowe, London, were used for confirmation of the K1 character and to select K$^-$ mutant derivatives of K1$^+$ strains. The antiserum and phages gave identical results with all strains tested, except for K92 strains, with which the antiserum reacted positively and the bacteriophages reacted negatively.

Isolation of traTp and raising of monoclonal antibodies. Whole-membrane preparations from bacteria of *E. coli* C600 Rif^r(pKT107) or C600 Rif^r(pKT146) were obtained and extracted with 2% Triton X-100, as described by Manning et al. (15). The Triton X-100–insoluble material was subse-

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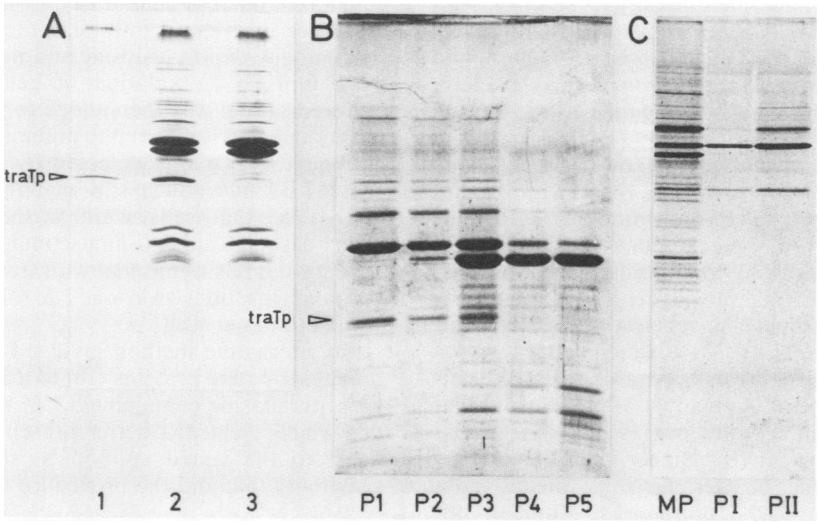

FIG. 1. Partial purification of traTp for immunization. Protein preparations were subjected to SDS-PAGE, and proteins were subsequently revealed by staining of gels with Coomassie blue. (A) Lanes: 1, traTp preparation used for immunization; 2, starting material, a Triton X-100–insoluble outer membrane protein preparation obtained from C600 Rif^r(pKT107) bacteria; 3, a similar preparation of C600 Rif^r(pACYC184) bacteria, which served as a *traT*$^-$ control. (B) Five pools from the first Sephacryl S-200 column (lanes: P1, fractions 155 to 159; P2, fractions 160 to 166; P3, fractions 167 to 176; P4, fractions 177 to 179; P5, fractions 180 to 185) were each concentrated to 500 μl and analyzed by SDS-PAGE. As can be seen, pools P1 and P2 contained ca. 50% of the traTp and were depleted of most of the OmpA protein and some low-molecular-weight proteins. These two pools were combined and used for a second Sephacryl S-200 run in the presence of 1% 2-mercaptoethanol (panel C). (C) Pool 1 (PI, fractions 165 to 170) and pool 2 (PII, fractions 171 to 180) after rechromatography of traTp in the presence of 2-mercapthoethanol. The first lane (MP) corresponds to a preparation of Triton X-100–insoluble outer membrane proteins, comparable to lane 2 in panel A. As can be seen, the second Sephacryl run resulted in a nearly complete separation of traTp from all low-molecular-weight and most high-molecular-weight contaminants, with the exception of one major outer membrane protein with an M_r of 3.7×10^4. Preparative PAGE of pool 1 yielded the material shown in lane 1 in panel A, that was used for immunization.

quently solubilized by incubation for 2 h at 37°C in 0.125 M Tris-hydrochloride (pH 6.8)–2% sodium dodecyl sulfate (SDS) and fractionated by gel filtration chromatography on a Sephacryl S-200 column (95 by 2.5 cm) equilibrated in Tris-glycine buffer (0.025 M Tris, 0.192 M glycine, pH 8.3) containing 1% SDS. The initial 90 ml of eluate was discarded after which 1-ml fractions were collected (about 150), and a portion of every fifth fraction was analyzed by electrophoresis on a 12 to 15% polyacrylamide-SDS gradient slab gel. Fractions containing traTp (Fig. 1B) were pooled and concentrated to 1 ml in a collodion bag and applied to a second Sephacryl S-200 column. The second Sephacryl run differed from the first only in that the sample and elution buffer contained 1% 2-mercaptoethanol. The eluted fractions were again analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and traTp-containing fractions 165 to 170 were pooled (Fig. 1C, lane PI) for further purification by preparative SDS-polyacrylamide gradient slab gel electrophoresis under the same conditions as those used for analytical runs. After electrophoresis, two marginal strips of the gel were cut off and stained, and the rest was frozen. The region of the gel containing the traTp band was then excised, homogenized, and extracted with four 10-ml volumes of buffer (0.05 M Tris acetate [pH 7.8], 0.01 M mercaptoethanol, 1% SDS) at 4°C over a period of 8 h (23). Urea was then added to a final concentration of 6 M, and the sample was concentrated to 500 μl in a collodion bag. Insoluble material was removed by centrifugation, and the supernatant fluid was finally dialyzed against 0.125 M Tris-hydrochloride, pH 6.8. The purity of the traTp obtained by this procedure is shown in Fig. 1A, lane 1. Note that this procedure gives relatively pure traTp in low yields; it is thus suitable for the rapid isolation of traTp in limited quantities, such as those required for immunization purposes, etc., but is less appropriate for large-scale purification of traTp.

Partially purified traTp (20 μg) was mixed with complete Freund adjuvant (Difco Laboratories, Detroit, Mich.) and injected intraperitoneally into 12-week-old BALB/c mice (Zentralinstitut für Versuchstierkunde, Hanover). Two intraperitoneal booster injections without complete Freund adjuvant were given 2 and 4 weeks later. The mice were killed 4 days after the final booster injection, immune serum was taken by heart puncture, and spleen lymphocytes were fused with the hybridoma line X63-Ag8.653 (12), as described previously (6). Supernatant fluids from the clones obtained were tested for their production of antibody against purified traTp in an enzyme-linked immunosorbent assay (ELISA) by means of peroxidase-labeled anti-mouse immunoglobulins (Dako, Munich) with ABTS [2,2′-arino-di-(3-ethyl-benzthiazoline-6-sulfonic acid)diammonium salt] (Sigma Chemical Co., Munich) as the substrate (24).

TraTp was fixed to poly-D-lysine–coated microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) by cross-linking with 0.01% glutaraldehyde. Membrane proteins from the TraT-negative strain C600 Rif^r(pACYC184), prepared from Triton X-100–insoluble outer membrane protein preparations by solubilization in SDS (2%), served as a control for specificity of antibody binding. Ascites for the bulk preparation of monoclonal antibodies were induced in Pristane-primed BALB/c mice. Immunoglobulin class specificity was determined in an ELISA with subclass-specific, peroxidase-labeled antibodies (Nordic, Tilburg, The Netherlands). The anti-OmpA protein-monoclonal antibody was raised in BALB/c mice by immunization with whole bacteria of *E. coli*

strain C600 Rif^r(pKT107) and identified by its reaction with the well-known heat modifiable OmpA protein in outer-membrane preparations of TraTp$^+$ and TraTp$^-$ bacteria and its lack of reaction with preparations from *ompA*$^-$ bacteria (kindly provided by U. Henning, Tübingen, Federal Republic of Germany).

SDS-PAGE and immunoblotting. Bacteria harvested from overnight cultures in TSB were washed twice with physiological saline and adjusted to a concentration of 5×10^8 cells per ml. After centrifugation, the cell pellet was dissolved in sample buffer (0.0625 M Tris-hydrochloride [pH 6.8], 5% 2-mercaptoethanol, 2% SDS, 12.5% glycerol) and heated to 100°C for 5 min. SDS-gradient slab gel electrophoresis (12 to 15%) of the solubilized proteins (13) was then carried out for 4 h in a Desaphor electrophoresis apparatus (Desaga, Heidelberg, Federal Republic of Germany). After electrophoresis, gels were either stained with Coomassie blue or transferred to nitrocellulose paper (Schleicher and Schüll, Inc., Dassel, Federal Republic of Germany) by means of a Transblot apparatus (Bio-Rad Laboratories, Munich). Immunological detection of traTp bands on nitrocellulose sheets was accomplished with either culture supernatants of hybridoma clones producing anti-traTp monoclonal antibodies or a 1:1,000 dilution of an ascites fluid. Bound monoclonal antibodies were subsequently revealed with peroxidase-labeled anti-mouse immunoglobulin G (IgG), using 4-chloro-1-naphthol as the substrate for the enzyme reaction. Densitometric analysis of the stained traTp bands on the nitrocellulose paper was performed with a model 1650 scanning densitometer (Bio-Rad).

ELISA for detection of traTp in clinical isolates. Bacteria were obtained and washed as described above and suspended in detergent solution (0.01 Tris-hydrochloride [pH 8.0], 5 mM EDTA, 0.1 M dithiothreitol, 2% SDS) to a concentration of 2.5×10^{10}/ml, and the cell suspension was heated to 100°C for 5 min and incubated for 1 h at 37°C. Dilutions of solubilized bacteria in 0.01 M phosphate-buffered saline, pH 7.3, were transferred into microtiter plates (20 μl per well) precoated with poly-D-lysine and were fixed with glutaraldehyde (0.01%). A solution of gelatin (2% in phosphate-buffered saline) was then added to the wells, and the plates were incubated for 20 min to minimize nonspecific binding of antibody. TraTp was detected by addition to the wells of Mo-414-B9 hybridoma supernatant fluid (20 μl per well); after 1 h, the wells were washed and developed with peroxidase-labeled anti-mouse immunoglobulin. This procedure gave a positive signal with traTp-producing bacteria at cell concentrations as low as 2.5×10^7 bacteria per ml or 5×10^5 bacteria per well.

An alternative method involved the transfer of enriched outer-membrane proteins (18) to a 2% solution of octyl-β-D-glycopyranoside containing 0.1 M dithiothreitol. This method, which yielded a better presentation of traTp determinants to the tested antibodies, showed a slightly higher sensitivity than did the procedure described above.

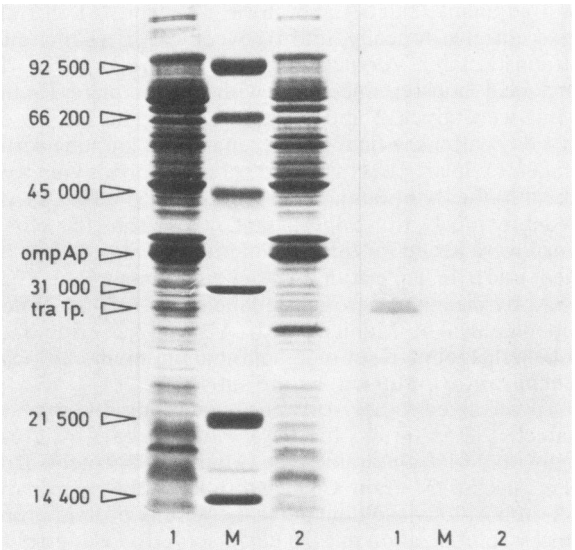

FIG. 2. Immunoblot of proteins of traTp$^+$ and traTp$^-$ bacteria with the anti-traTp monoclonal antibody. On the left is the Coomassie blue stained SDS-polyacrylamide gel, and on the right is an immunoblot of the same gel. Lanes: 1, 10^7 solubilized bacteria of strain C600(pKT107); 2, 10^7 bacteria of strain C600(pACYC184); M, marker proteins (Bio-Rad). In addition to the marker proteins, traTp in lane 1 and OmpA protein in lanes 1 and 2 are indicated by arrows.

RESULTS

Generation and characterization of an anti-traTp monoclonal antibody. Immunization of BALB/c mice with living bacteria expressing the plasmid-encoded traTp, or with outer-membrane preparations of these bacteria, and the subsequent production of hybridomas did not yield a single clone producing an anti-traTp monoclonal antibody from more than 2,000 tested, derived from three cell fusions. We therefore purified traTp to the extent seen in Fig. 1, immunized with this partially purified material, and obtained several monoclonal antibodies specifically reacting with traTp, as shown, for example, for monoclonal antibody Mo-414-B9 by immunoblotting (Fig. 2) and in a traTp-specific ELISA (Fig. 3). A second monoclonal antibody (Mo-759-G8) induced by immunization with whole bacteria that reacted with a major outer membrane protein (the heat modifiable OmpA protein) present in both TraT$^+$ and TraT$^-$ strains was used as a control in the ELISA.

Antibody Mo-414-B9 is an IgG of subclass gamma 1, having kappa light chains. It binds protein A weakly and does not bind to TraT$^+$ whole bacteria, not even to bacteria carrying the pKT146 plasmid, which contain more than 100,000 copies of traTp per cell (16). However, the antibody readily detected traTp in bacteria solubilized with SDS or nonionic detergents and in isolated and solubilized outer-membrane preparations; this indicates that the traTp antigenic determinant detected by monoclonal antibody Mo-414-B9 is not accessible on living bacteria and is therefore masked or buried within the membrane.

The sensitivity of the test systems for traTp, using a $1{:}10^3$ dilution of ascites fluid of Mo-414-B9, was high enough to detect traTp in 10^7 bacteria by immunoblotting (Fig. 2) and in 5×10^5 bacteria by the ELISA (Fig. 3).

Use of the monoclonal antibody to screen for the presence of traTp in clinical isolates. By means of the monoclonal antibody and the immunoblot system, we examined about 400 clinical isolates of *E. coli* for the presence of traTp. As can be seen in Table 1, although traTp was found in 38% of nonclinical (fecal) isolates of *E. coli*, it was found in a significantly higher proportion of clinical isolates of *E. coli*, namely in 56% of sepsis strains and in 51% of invasive urinary-tract strains. Moreover, of 22 *E. coli* isolates from children with infantile diarrhea, typed with regard to O and K antigens and classified as enteropathogenic, 16 (i.e., 73%) were traTp-positive. The traTp was found in an even greater proportion (66 to 88%) of *E. coli* strains that produced the K1

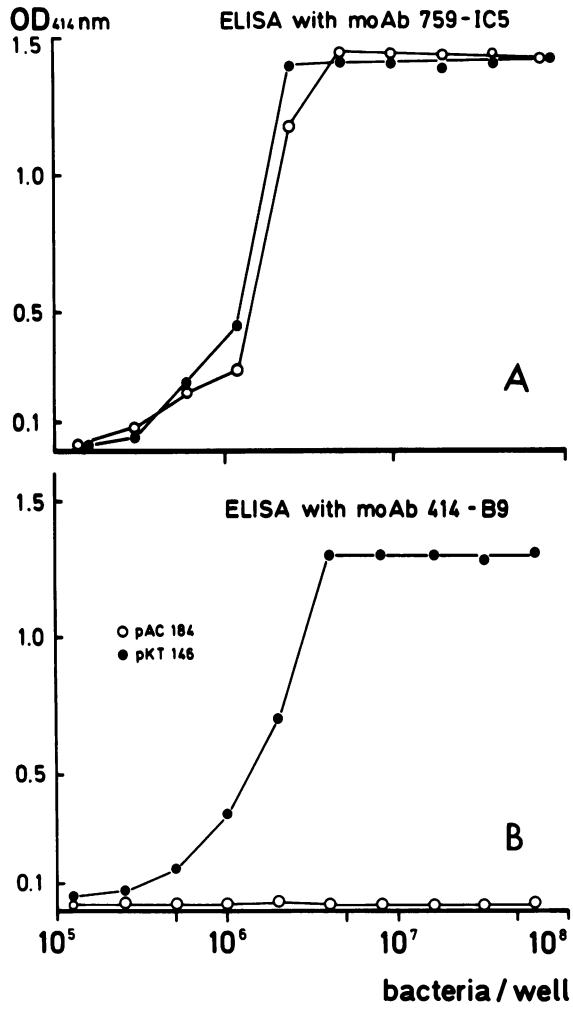

FIG. 3. ELISA for detection of traTp. The abscissa shows the absolute number of solubilized bacteria, either C600(pACYC184) or C600(pKT146), used for coating of the microtiter wells, whereas the ordinate shows the absorbance at 414 nm produced by the peroxidase reaction. (A) ELISA reaction with monoclonal antibody Mo-759-G8 that binds to a major outer membrane protein (OmpAp) present in both strains. (B) ELISA reaction with the anti-traTp monoclonal antibody Mo-414-B9.

capsule, a well-known pathogenesis factor (Table 2; see also Fig. 4). Again, traTp was found in a significantly higher proportion of $K1^+$ clinical isolates, namely in 88% of sepsis isolates and in 78% of UTI isolates, than in $K1^+$ fecal strains (66%). From 15 additional $K1^+$ isolates of UTI origin, 11 (73%) were traTp positive.

Comparison of the intensity of traTp bands, as determined from densitometer tracings of immunoblot filters of the reference strain C600 Rif^r(pKT107), which contains ca. 20,000 copies of traTp per cell (18), with those of the clinical isolates gave a rough estimate of 5,000 to 10,000 copies of traTp per cell in most of the latter traTp-positive isolates (e.g., Fig. 5A). This probably reflects the fact that $traT^+$ plasmids in *E. coli* are low-copy-number replicons, whereas the pKT107 plasmid is multicopy (M. A. Montenegro, D. Bitter-Suermann, M. E. Agüero, F. C. Cabello, and K. N. Timmis, manuscript in preparation).

It should be noted that, whereas only a single traTp band of M_r 2.8×10^4 was revealed by the immunoblot technique when TSB-grown cells of the K-12 laboratory strain or of clinical isolates of *E. coli* were analyzed, a double band was reproducibly observed when colonies from blood agar plates containing 5% sheep erythrocytes were analyzed (Fig. 5B). Although this double-band pattern needs to be characterized further, it is highly reminiscent of that seen with protein preparations containing traTp precursor (9). The second traTp band, which had an M_r 10^3 greater than that of the usual traTp species, was presumably produced in response to bacterial growth on rich medium.

TABLE 1. Incidence of traTp in *E. coli* isolates as detected by immunoblot analysis[a]

Type of sample	Total no. of isolates tested	% traTp positive
Sepsis[b]	153	56
UTI[c]	100	51
Fecal[d]	100	38

[a] Statistical analysis of traTp positivity by the chi-square test showed a significant increase of traTp in the clinical isolates (χ^2: $P < 0.01$).
[b] From a total of 254 positive blood cultures containing gram-negative pathogens (mostly *Enterobacteriaceae*).
[c] Isolates from upper UTI with significant bacteriuria (10^5 *E. coli* per ml of urine).
[d] Isolates from feces of normal, healthy persons.

DISCUSSION

The results reported here reveal the production of the traTp in a significantly higher proportion of clinical isolates of *E. coli* (51 to 56%) than in normal fecal isolates (38%). Even more remarkable was the high degree of association between production of traTp and elaboration of the K1 capsule (66 to 88%), a known virulence determinant of many pathogenic strains of *E. coli* (2, 20), and between production of traTp and enteropathogenicity (73%). Southern blot analysis of representatives of the groups of strains in Table 1, with a *traT* gene-specific DNA fragment, has shown that traTp is in all cases coded by plasmids, largely IncF group plasmids (Montenegro, Bitter-Suermann, Agüero, Cabello, and Timmis, in preparation). This suggests that traTp, or another factor encoded by $traT^+$ plasmids, contributes to the virulence of a high proportion of pathogenic strains of *E. coli*. A similar association of the carriage of ColV plasmids (IncF group) by K1-positive clinical strains of *E. coli* has also been reported (3). Although traTp has been shown to mediate bacterial resistance to complement and phagocytosis in laboratory strains of *E. coli*, its role if any in the pathogenicity of clinical isolates is likely to be dependent upon other factors, such as capsular polysaccharides, lipo-

TABLE 2. Incidence of traTp in K1- and nonK1-producing *E. coli* isolates as detected by immunoblot analysis[a]

Type of sample	No. (%) of $K1^+$ isolates		No. (%) of $K1^-$ isolates	
	TraTp positive	TraTp negative	TraTp positive	TraTp negative
Fecal	8 (66%)	4 (34%)	30 (34%)	58 (66%)
UTI	11 (78%)	3 (22%)	41 (48%)	45 (52%)
Sepsis	15 (88%)	2 (12%)	71 (52%)	65 (48%)

[a] χ^2 (Omnibus Test by Le Roy): $P < 0,005$ *E. coli* isolates previously tested (Table 1) were further subdivided into K1-positive ($K1^+$) and K1-negative ($K1^-$) strains and analyzed for traTp. Absolute numbers of each type of isolate are given.

polysaccharides, and plasmid-encoded proteins other than traTp (2, 5, 10, 18, 21).

To determine whether traTp plays a role in pathogenesis, it will be necessary to construct *traT*$^-$ deletion derivates of several *traT*$^+$ pathogenic strains of *E. coli* and to compare the virulence of the traTp$^+$-traTp$^-$ pairs (that are isogenic for all other characters) in a suitable animal model. If these experiments demonstrate that traTp contributes to bacterial virulence, the traTp monoclonal antibody will clearly constitute a useful reagent for epidemiological studies. Alternatively, even though traTp itself may not directly contribute to the virulence of clinical isolates, its easy detection by the methods described above makes it a useful marker for F-like plasmids which may code for other factors of pathogenicity and antibiotic resistance in *E. coli*. The two methods used here to detect traTp each have a specific advantage. The ELISA is more rapid and more sensitive and detected traTp in all isolates that were shown by immunoblotting to be traTp$^+$. The immunoblotting procedure not only detects the production of traTp but also identifies it as a specific-molecular-weight species of protein. In an initial study like the one described here, or in an analysis of traTp in non-*E. coli* isolates, the additional information provided by the immunoblotting procedure is important. Indeed, recent experiments have revealed the production in isolates of certain non-*E. coli* members of the family *Enterobacteriaceae* of an anti-traTp antibody-reacting species having a molecular weight higher than that of traTp itself (M. Jürs, H. Peters, K. N. Timmis, and D. Bitter-Suermann, manuscript in preparation).

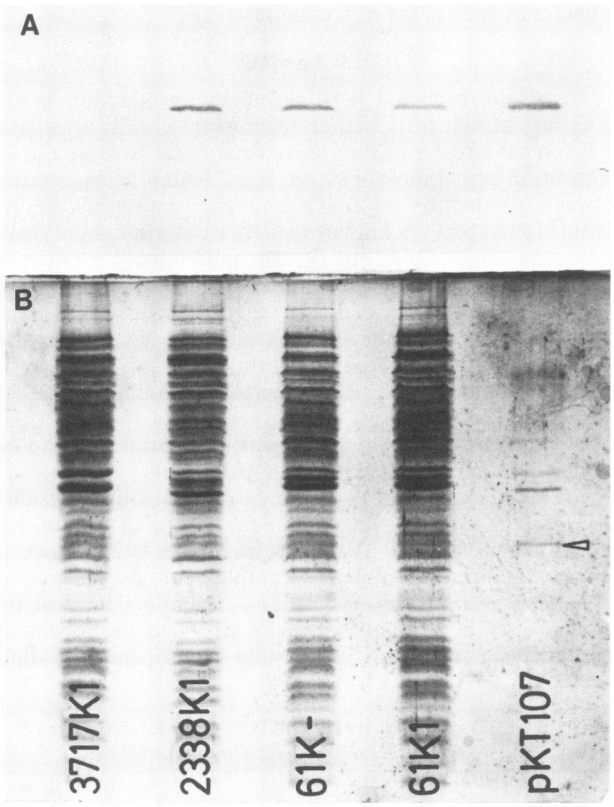

FIG. 4. Immunoblot of solubilized *E. coli* sepsis strains. 10^7 bacteria of K1$^+$ strains 61K1, 2338K1, and 3717K1 and a K1$^-$-derivative of 61K1 were solubilized and applied to the gel. A 1:20 dilution of a membrane preparation of C600(pKT107) was used as a reference. As can be seen, 61K1, 61K$^-$ and 2338K1 were traTp positive, whereas strain 3717K1 was traTp negative. The differences in the protein patterns of the different strains reflect stable differences in the compositions of their outer membrane proteins. (A) Immunoblot; (B) SDS-PAGE stained with Coomassie blue.

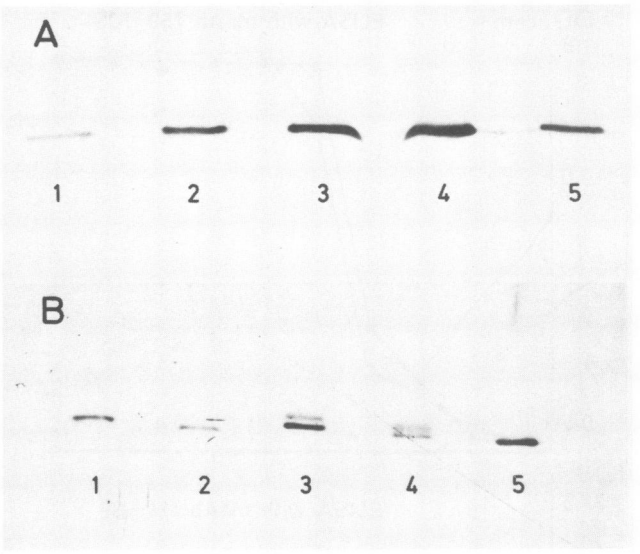

FIG. 5. Quantitation of traTp in clinical isolates and effect of growth medium on traTp species produced. (A) Amount of traTp produced by a UTI strain of *E. coli*. Tracks 1 to 4: 2.5×10^6, 5×10^6, 1×10^7, and 2×10^7 TSB-grown bacteria of strain C600 Rif^r(pKT107), which contains 2×10^4 traTp copies per cell (18); track 5, 1×10^7 TSB-grown bacteria of a UTI isolate. The intensity of traTp in track 5, as calculated from the peak area of the densitometrically analyzed immunoblot bands, is the same as that in track 2; this particular UTI strain therefore contains 1×10^4 traTp copies per cell. (B) Demonstration of double bands of traTp extracted from blood agar-grown bacteria. Track 1, TSB-grown bacteria of a UTI strain; track 2, bacteria of a TSB-grown sepsis strain; tracks 3 and 4, blood agar plate-grown bacteria of the same strains shown in tracks 1 and 2 but grown on blood; track 5, 10^7 TSB-grown bacteria of C600 rif^r(pKT107).

The reason that we were not able to induce monoclonal antibodies against traTp, when we used whole bacteria or intact outer membranes for immunization, remains unclear considering that traTp is reported to be a major outer-membrane protein which is exposed on the cell surface (15, 22). The anti-traTp monoclonal antibody Mo-414-B9 and two other monoclonal antibodies with a similar reaction pattern, which were induced by immunization with partially purified traTp, efficiently detect solubilized traTp but not native traTp on whole cells. Therefore, the antibody most probably detects an antigenic determinant hidden within the membrane, which is unfolded or unmasked by treatment of cells with detergents. If further attempts fail to produce an anti-traTp antibody that reacts with whole cells, the possibilities must be considered that exposed domains of traTp, which may be identified from the available nucleotide sequence (19), are weakly immunogenic or that traTp is not as exposed on the cell surface as has been suggested by previous experiments involving the radiolabeling of traTp on whole cells by lactoperoxidase coupling of ^{125}I (15, 22) and by the surface exclusion phenomenon (1, 15).

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