2F3 Monoclonal Antibody Recognizes the O26 O-Antigen Moiety of the Lipopolysaccharide of Enterohemorrhagic *Escherichia coli* Strain 4276

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Enterohemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC) organisms are groups of pathogenic strains whose infections are characterized by a typical lesion of enterocyte attachment and effacement. They are involved in enteric diseases both in humans and in animals, and EHEC strains can be responsible for hemolytic uremic syndrome in humans. Previously, it was shown that the 2F3 monoclonal antibody (MAb) is specific for the O26 EHEC and EPEC strains (P. Kerr, H. Ball, B. China, J. Mainil, D. Finlay, D. Pollock, I. Wilson, and D. Mackie, Clin. Diagn. Lab. Immunol. 6:610–614, 1999). As these groups of bacteria play an important role in pathology, the aim of this paper was to characterize the antigen recognized by the 2F3 MAb and its genetic determinant. A genomic locus containing the entire O-antigen gene cluster from an O26 EHEC strain was shown to be sufficient for the production of the antigen recognized by the 2F3 MAb in an *E. coli* DH5α strain. By transposon mutagenesis performed on the recombinant plasmid, all 2F3 enzyme-linked immunosorbent assay-negative mutants had their transposons inserted into the O-antigen gene cluster. The O-antigen gene cluster was also cloned from an O26 EHEC strain into the *E. coli* DH5α strain, which then produced a positive result with the 2F3 MAb. Further analysis of the type of lipopolysaccharides (smooth or rough) produced by the clones and mutants and of the O antigen of the 2F3-positive clones confirmed that the epitope recognized by the 2F3 MAb is located on the O antigen in the O26 EHEC and EPEC strains and that its genetic determinant is located inside the O-antigen gene cluster.

Enterohemorrhagic *Escherichia coli* (EHEC) strains are an important class of pathogens for humans and animals. The term EHEC denotes *E. coli* strains that cause attaching and effacing lesions on epithelial cells, express Shiga toxin-encoding genes, possess a 60-MDa virulence plasmid, and produce hemorrhagic colitis and/or hemolytic uremic syndrome (17, 18).

Besides those of the O157:H7 and O157:H− serotypes, EHEC strains of serogroup O26 are the most common strains found in human infections (4, 17). In addition, O26 EHEC strains are an important cause of enteritis in calves (12). One of the most important steps in the pathogenic mechanism of enteric bacteria is the initial adhesion of the bacteria to the intestinal wall. Several stages of colonization have been recognized in attaching and effacing *E. coli* pathogenesis, some of which await characterization. Some level of host specificity is shown by EHEC strains, but the role in host specificity of the recognized virulence factors, such as Shiga toxin 1 (Stx1) and/or Stx2 and the intimin protein (a protein that is encoded by the eae gene and necessary for the production of the attaching and effacing lesion), is unknown (8, 19). The bovine host specificity shown by O26 EHEC and enteropathogenic *E. coli* (EPEC) strains could be based on the production of a colonization factor, such as an adhesin, that is specific for cattle; such a colonization factor has been demonstrated to be the basis of the host specificity of classical human EPEC strains. Such an adhesin is likely to be a specific immunogen exposed at the bacterial cell surface (3, 13, 15, 17, 23, 24).

A monoclonal antibody (MAb), 2F3, was derived against a surface antigen extracted from the bovine O26 EHEC strain 4276; it tested positive in an enzyme-linked immunosorbent assay (ELISA) with all of the O26 and half of the O111 bovine and human EHEC and EPEC strains (9). Recent reexamination of six of the O111 EHEC and EPEC strains that tested positive revealed a contamination by O26 EHEC or EPEC strains. Purification of the strains in the mixed culture demonstrated that the O111 strains were 2F3 negative and the O26 strains were 2F3 positive (J. McCappin and H. Ball, unpublished data).

Since the 2F3 MAb is specific for the O26 EHEC and EPEC strains, it may not only represent a diagnostic tool of these confirmed pathogens but also recognize a factor involved in the production of the attaching and effacing lesion. In view of these results, this study was initiated (i) to characterize the antigen recognized by the 2F3 MAb and its genetic determinant and (ii) to reexamine the specificity of the 2F3 MAb within the O26 serogroup.

**MATERIALS AND METHODS**

**Bacterial strains and media.** The O26 EHEC strain 4276, isolated from a calf with a case of enteritis in Northern Ireland, was used to characterize the genetic
basis of the antigen recognized by the 2F3 MAb (2F3 antigen). This strain was characterized to be eae and xfa positive and to produce a complete lipopolysaccharide (LPS) with an O antigen belonging to the O26 serotype (9). The DH5α K-12 derivative E. coli strain was used as the host strain for the cloning and mutagenesis assays. It possesses the O-antigen (rfa) gene cluster for the production of an O16-type O antigen but produces an LPS without the O antigen and rough-type colonies on solid medium as a consequence of two independent mutations in the rfb gene cluster. The first mutation (the rfa-50 mutation) consists of an IS5 insertion at the 3’ end of the rfa gene cluster and is representative of most K-12 derivatives. The second mutation (the rfa-51 mutation) consists of a deletion at the 5’ end of the rfa gene cluster (11). A collection of 70 O26 EPEC or EHEC strains, 7 O26 non-EPEC and non-EHEC strains, and 3 E. coli strains belonging to other serogroups were used to reexamine the specificity of the 2F3 MAb. The strains were isolated from humans or animals, either with diarrhea disease or hemolytic uremic syndrome or without clinical signs, in Europe and North America (Table 1).

### Table 1. E. coli strains used in this study and their 2F3 ELISA results

| Strain | Pathotype | Serotype | 2F3e | eae | O26e | Source |
|--------|-----------|----------|------|-----|------|--------|
| HS     | K-12      | O9:H4    | –    | –   | –    | Human  |
| E2348  | EPEC      | O127     | –    | +   | –    | Human  |
| H511   | EHEC      | O102     | –    | +   | –    | Human  |
| 193    | EHEC      | O26      | +    | +   | +    | Cattle |
| 4276   | EHEC      | O26      | +    | +   | +    | Cattle |
| 3578E9 | EHEC      | O26:H11  | +    | +   | +    | Cattle |
| 379S89 | EHEC      | O26:H11  | +    | +   | +    | Cattle |
| 63IKH91| EHEC      | O26      | +    | +   | +    | Cattle |
| 122    | EHEC      | O26      | +    | +   | +    | Cattle |
| 63     | EHEC      | O26      | +    | +   | +    | Cattle |
| A39    | EHEC      | O26      | +    | +   | +    | Cattle |
| A14    | EHEC      | O26      | +    | +   | +    | Cattle |
| 3953   | EPEC      | O26      | +    | +   | +    | Cattle |
| C15333 | EPEC      | O26      | +    | +   | +    | Cattle |
| 333K1H | EPEC      | O26      | +    | +   | +    | Cattle |
| 33IKH91| EPEC      | O26      | +    | +   | +    | Cattle |
| 35IKH91| EPEC      | O26      | +    | +   | +    | Cattle |
| TC3108 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3109 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3117 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3180 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3269 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3273 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3302 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3305 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3375 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3380 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3629 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3630 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3631 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3632 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3656 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3657 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC3748 | EHEC      | O26      | +    | +   | +    | Cattle |
| TC6169 | EHEC      | O26:H11  | +    | +   | +    | Cattle |
| TC659  | EPEC      | O26:HNM  | +    | +   | +    | Cattle |
| TC1988 | EPEC      | O26:HNM  | +    | +   | +    | Cattle |
| TC3145 | EPEC      | O26      | +    | +   | +    | Cattle |
| TC3486 | EPEC      | O26      | +    | +   | +    | Cattle |
| TC3848 | EPEC      | O26:H11w | +    | +   | +    | Cattle |

### Construction of a DNA genomic library

The cosmid library was obtained from the O26 E. coli strain 4276 (9) by using the Expand cloning kit (Roche, Mannheim, Germany). Briefly, total genomic DNA, extracted with an AquaPure genomic DNA purification kit (Bio-Rad), was partially digested by serial dilution at 37°C with EcoRV (Invitrogen) in order to generate blunt-end fragments compatible with the linearized vector. Following the inactivation of the enzyme at 65°C for 15 min, fragments approximately 10, 20, and 30 kb in length were inserted into three pHC79 cosmid derivative vectors with a cloning capacity of 5 kb. The constructs were packaged into a lambda bacteriophage and then transfected into a DH5α E. coli magnesium culture, according to the manufacturer’s instructions. Transformants were selected on LB plates containing ampicillin, and the 2F3 antigen-positive clones were identified by an ELISA.

### In vitro mutagenesis

Independent mutations in the recombinant plasmid from the 2F3 ELISA-positive clone were performed by an in vitro insertion strategy using the EZ::TN <KAN>2> insertion kit (Epipertec). The kit uses the benefit of the Tn5 transposon system. The transposon containing the kanamycin resistance gene is randomly inserted into the target DNA under the control of transposase that is separately added to the reaction mix. After the inactivation of
the transposase, the transposon can no longer move from its insertion site. The target DNA was the cosmid DNA purified from the 2F3 ELISA-positive clone with the plasmid mini kit (QIAGEN, Westburg, Germany). After a 2-h insertion reaction at 37°C, the reaction mix (the target DNA, the EZ::TN <KAN2>- transposon, and EZ::TN transposase in corresponding buffer) was incubated for 10 min at 70°C to inactivate the transposase and dialyzed for 60 min against distilled water. From the mix, 0.04 μg of DNA was electrophoresed at 2.25 V, 40 μF, and 132 Ω into E. coli DH5α electrocompetent cells (Invitrogen). After incubation for 45 min at 37°C, the transformants were plated onto Luria agar plates containing kanamycin for selection.

ELISAs. An ELISA with the 2F3 MAb was used to screen the cosmid and mutant libraries for 2F3-positive and 2F3-negative clones, respectively, and all the strains from the collection. An ELISA with the anti-O26 E. coli agglutinating serum (Bio-Rad) was used to serotype the 2F3 ELISA-positives clones. Assays were performed with 96-well microtiter plates (Greiner) as previously described (9).

The absorbance was measured at 450 nm with an ELISA plate reader (Bio-Rad). The strains 4276 and DH5α were used as positive and negative controls, respectively. The 2F3 ELISA-positive cosmid clone used in the mutagenesis assay was also used as a positive control to screen the mutant library. Optimum dilution of the reagents was obtained by titration. Readings three times higher than the average negative-control value were considered positive reactions.

LPS production and serotyping assays. The presence of a complete LPS (with O antigen) or of an incomplete LPS (without the O antigen) was detected by staining distinct colonies by the crystal violet staining assay (22). The smooth colonies are not stained since the presence of the O antigen on the LPS prevents staining distinct colonies by the crystal violet staining assay (22). The smooth colonies were stained blue, whereas the rough colonies, because of the absence of the O antigen on the LPS, take up the dye and stain blue.

Serotyping was performed on wild-type strains, clones, and mutants in an agglutination assay using antiserum from Laboratorio de Referencia de E. coli K-12. Polyacrylamide gel electrophoresis was carried out on a whole-cell preparation from the wild-type O26 EHEC (4276) and EPEC (C15333) strains and from the clones obtained by restriction (IXD2) and long-range PCR (III7) using NuPAGE 4 to 12% gradient gels under reducing conditions (Novex; Invitrogen) according to the manufacturer’s instructions. Gels were stained with SimplyBlue SafeStain (Invitrogen) or transferred onto a nitrocellulose membrane by blotting at 30 V for 1 h. After the transfer and blocking stages, the membranes were probed either with the 2F3 MAb or with the anti-O26 immune serum (Bio-Rad) as primary antibody. Specific immunoglobulin peroxidase conjugate was used as a secondary antibody (Sigma). The peroxidase substrate was 4-chloro-1-naphthol (Sigma), and after 10 min of incubation, the membrane was washed with distilled water to stop any further reaction.

RESULTS

Cosmid library screening. A cosmid library of more than 2,000 clones was obtained from strain 4276. One of them (IXD2) was ELISA positive with the 2F3 MAb (Table 3). The length of the insert was estimated, in a 0.5% agarose electrophoresis gel, to be approximately 30 kb.

Both the M13 FP and M13 RP primers were used to screen the two extremities of the insert. The sequence obtained with the M13 RP primer showed 95% identity (129 of 135 bp) with the ugd gene that codes for UDP-glucose 6-dehydrogenase and is found on existing genomic maps of both E. coli O157:H7 and E. coli K-12. The sequence obtained with the M13 FP primer showed 97% identity (216 of 222 bp) with the wcaF gene, which codes for a putative acetyltransferase also found on existing genomic maps of both E. coli O157 and E. coli K-12.

### TABLE 2. Sequences of the primers used in this study

| Primer name | Primer sequence | Reference |
|-------------|-----------------|-----------|
| gnd | 5'-CQCTGGCATAACGACGACCGCAGTCTCGTCTG-3' | 2 |
| galF | 5'-ACCTGACAACAAGCAGCTTCATACAC-3' | Epicentre |
| Kan2 FP | 5'-GCAAATTTAACATCAGAGATTGTTG-3' | Epicentre |
| Kan2 RP | 5'-GGGAAACAGCAGCCAG-3' | Invitrogen |
| M13 FP | 5'-GAAAAGCAGGCG-3' | Invitrogen |
| M13 RP | 5'-CAGGAAACACGCTATGAC-3' | Invitrogen |
| wbuA FP | 5'-CTCTGATTATACAGAAGCA-3' | 7 |
| wbuA RP | 5'-ATGTGAATTATAGCGTTCTT-3' | 7 |
PCR for an O26-specific gene.

polyclonal immune serum to O26 (from Bio-Rad and LREC) and O16 (from LREC); O26 ELISA, O26 ELISA with polyclonal immune serum to O26 (Bio-rad); insertion site.

(E4, F5, F6, H2, and B1) obtained by insertional mutagenesis from the clone IXD2. The arrowheads target the mutated gene and not the actual insertion site.

In vitro mutagenesis. In order to identify the genetic determinant of the antigen recognized by the 2F3 MAb, in vitro insertional mutagenesis was performed with the recombinant plasmid from the 2F3 ELISA-positive clone. More then 1,400 mutants were obtained. After this mutant library was screened by ELISA with the 2F3 MAb, more than 100 mutants were obtained. After this mutant library was screened for the 2F3 ELISA-positive clones, and the mutants

mutants, the transposons had been inserted inside the O-antigen gene cluster at different positions (Fig. 1).

For three of the mutants (F5, F6, and H2), the transposon was inserted at similar locations, approximately 5 kb from the gnd gene and approximately 8 kb from the galF gene. The distance between the sites of insertion of the transposons in the mutants F5 and F6 was 31 bp, and similarly, the sites of insertion of the transposons in the mutants F6 and H2 were 174 bp apart.

In the E4 mutant, the transposon was inserted approximately 6 kb from the gnd gene and 7 kb from the galF gene. In the B1 mutant, the transposon was inserted 0.8 kb from the gnd gene and approximately 12 kb from the galF gene.

Cloning the O-antigen gene cluster. The product of the long-range PCR using the galF and gnd primers from strain 4276 was cloned into the E. coli strain DH5α. This recombinant clone (IIIC7) tested positive in the ELISA with the 2F3 MAb and with the anti-O26 immune serum (Table 3).

O-antigen production and serotyping assays. In order to study the relationship between the O26 antigen and the 2F3 character, the production of the O antigen was examined by using the crystal violet staining assay (Table 3). The 2F3 ELISA-positive clones (IXD2 and IIIC7) and the wild-type 2F3 ELISA-positive strains (4276 and C15333) produce a smooth-type LPS (with the O antigen). Host strain (E. coli DH5α) and all 2F3 ELISA-negative mutants produce a rough-type LPS (without the O antigen). The antigenic specificity of the O antigen was confirmed by an agglutination assay and by an ELISA. The 2F3 ELISA-positive strains and clones tested positive with the O26 immune serum, but the IXD2 clone

| Test | O26 wild-type E. coli strain: | Control strain serotype: |
|------|------------------------------|-------------------------|
| 2F3  | +                            | O26                     |
| LPS  | +                            | O16                     |
| O26  | +                            |                          |
| O26 ELISA | +                    |                          |
| O16  | +                            |                          |
| wbuA | +                            |                          |

a 2F3, ELISA with the 2F3 MAb as the primary antibody; LPS, LPS production checked by the crystal violet staining method; O26 and O16, agglutination with polyclonal immune serum to O26 (from Bio-Rad and LREC) and O16 (from LREC); O26 ELISA, O26 ELISA with polyclonal immune serum to O26 (Bio-rad); wbuA, PCR for an O26-specific gene.

b Clone obtained by restriction of genomic DNA.

c Clone obtained by long-range PCR.

![Fig. 1. Schematic representation of the insertions of the transposon EZ::TN <kan-2> within the O26-antigen gene clusters in the mutants (E4, F5, F6, H2, and B1) obtained by insertional mutagenesis from the clone IXD2. The arrowheads target the mutated gene and not the actual insertion site.](http://cvi.asm.org/Downloaded from)
tested negative in the agglutination assay and with the O16 serum. Conversely, the 2F3 ELISA-negative strain and mutants gave negative results with both immune sera. Nevertheless, in a PCR using primers for a specific gene of the O26 O-antigen gene cluster (wbuA), positive results were obtained with all 2F3 ELISA-positive and -negative wild-type strains, clones, and mutants (Table 3).

**Testing the specificity of the 2F3 MAb.** The 2F3 MAb gave positive reactions in the ELISA with all 70 eae-positive *E. coli* strains belonging to the O26 serogroup in a slide agglutination assay (Table 1). Conversely, the seven eae-negative O26 *E. coli* and the three eae-positive or eae-negative non-O26 *E. coli* strains tested negative in the ELISA with the 2F3 MAb. One 2F3 ELISA-positive strain, one negative wild-type O26 strain, and two positive clones (IXD2 and IIIC7) gave almost identical results by Western blotting with the 2F3 MAb and the O26 immune serum (Fig. 2). The only difference was the absence of a reaction of the 2F3 ELISA-negative wild-type O26 strain with the 2F3 MAb. The molecular weights of the bands from the 2F3 ELISA-positive wild-type strains and the IXD2 clone were the only other noticeable differences (Fig. 2).

**DISCUSSION**

The fact that related EPEC and EHEC strains are pathogens for humans and animals is well recognized. There is a diversity of strains that are pathogens for humans and animals, and even inside the same serogroup, the strains found as pathogens in human diseases are often different from those found in animal diseases, which shows that they have host specificity. This host specificity demonstrates a need to classify the strains into different groups according to their properties and their pathogenicity factors. Similarly, it is a necessity for assays to provide the tools to differentiate these strains.

To obtain a better understanding of the specificity of the 2F3 MAb for the O26 EHEC and EPEC strains, the antigen recognized by the 2F3 MAb and its genetic determinant were characterized. In this study, a recombinant plasmid carrying the genetic basis of the antigen recognized by the 2F3 MAb was obtained. The length of the cloned chromosomal DNA fragment was estimated to be about 30 kb. According to the sequences of the extremities of this fragment, its position on the existing *E. coli* maps corresponds to two gene clusters, the complete O-antigen gene cluster (22) and half of the colanic acid gene cluster from open reading frame 9 to open reading frame 21 (21). Both of these clusters are involved in LPS biosynthesis pathways, and based on information available from existing genomic maps for *E. coli* K-12 and *E. coli* O157: H7, the length of the fragment could be 30.5 kb with 27 genes or 33.7 kb with 28 genes, respectively (1).

By long-range PCR assays, it was shown that the fragment from the recombinant plasmid contains the entire O-antigen gene cluster from EHEC strain 4276. Furthermore, the sequences obtained after a mutagenesis assay showed a high degree of identity with genes involved in LPS biosynthesis pathways. The fnl-1 gene is one of the three genes that seem to be responsible for the synthesis of N-acetyl-L-fucosamine, one of the sugars composing the O26 O antigen (7). The wbuB gene from *Pasteurella multocida* and *Pseudomonas aeruginosa* and the rfb gene cluster of *L. interrogans* are also involved in the O-antigen biosynthesis pathway (6, 16). The wbuA and wbuB genes belong to the O-antigen gene cluster from the O26:K60: H311b *E. coli* strain and code for a putative rhamnosyl transferase and a putative t-L-fucosamine transferase, respectively (7).

Further analysis concerning the physical position of the transposons in the recombinant plasmid showed that all the 2F3 ELISA-negative mutants had inserted the transposon inside the O-antigen gene cluster. The F5, F6, and H2 mutants had inserted the transposon into the fnl-1 gene, the E4 mutant inserted it into the wbuA gene, and the B1 mutant inserted it into the wbuB gene.

The relationship between the character of 2F3 and LPS was clarified by the crystal violet staining of the colonies. By cloning a 30-kb fragment containing the entire O-antigen gene cluster from an EHEC strain into a rough-type LPS strain which is 2F3 ELISA negative, a 2F3 ELISA-positive clone with a smooth-type LPS was obtained. After in vitro insertional mutagenesis from the 2F3 ELISA-positive clone, several 2F3 ELISA-negative mutants were obtained, and all mutants produced a rough-type LPS. These results demonstrated the positive linkage between the character of 2F3 and the production of a smooth-type LPS. Moreover, the LPS produced carries the O26 O antigen recognized in agglutination assays, ELISAs, and Western blot assays with the two 2F3 ELISA-positive clones obtained after genomic DNA digestion and long-range PCR. Only the clone obtained after genomic DNA digestion did not react with the anti-O26 immune serum in the agglutination assay. One explanation could be that the complementation of the two mutations within the O-antigen gene cluster of the host strain, DH5α, produces an O antigen that is recognized by the 2F3 MAb but by neither the anti-O26 immune serum nor the anti-O16 immune serum. Alternatively, the presence in this clone of the second half of the colanic acid gene cluster, which is involved in LPS biosynthesis, may affect the agglutination result. This hypothesis is supported by the observation that the

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**FIG. 2.** Nitrocellulose immunoblot using 2F3 MAb and the anti-O26 immune serum (Bio-Rad) comparing whole-cell preparations of *E. coli* strains 4276 (lanes 2 and 7) (O26; eae and verotoxin positive), DH5α as a negative control (lanes 3 and 8), clone IXD2 (lanes 4 and 9), and T282 (O26; eae and verotoxin negative). Lanes 1 and 6 contain the benchmark prestained molecular weight markers (Invitrogen).
2F3 ELISA-positive clone obtained after PCR, which contains only the O-antigen gene cluster as the insert, is positive in the agglutination assay. So the presence of the disrupted colanic acid gene cluster may have resulted in the production of a hybrid LPS of a different size, as was observed by Western blotting, with interference in the agglutination assay.

In conclusion, the target of the 2F3 MAb is the O26 antigen from the EHEC and EPEC strains, and its genetic determinant is located inside the O-antigen gene cluster. The epitope recognized by the 2F3 MAb on the O26 O antigen has still not been identified. The O antigens are heterogeneous due to their sugar compositions, either monomers or polymers, and the linkage between them. The O26 O antigen contains three sugars: l-rhamnose, N-acetyl-t-fucosamine, and N-acetyl-d-glucosamine (14). The 2F3 MAb may recognize one of these sugars, a particular linkage, or a particular arrangement between them. Transposons in two of the 2F3 ELISA-negative mutants were inserted in genes involved in sugar transferase synthesis (wbuA and wbuB), resulting in the absence of the O antigen on the surfaces of these mutants. It is possible that these two genes have a role in the production of the O antigen by acting during the process of the assemblage of the O unit. On the other hand, the mutations in the fnt-l gene may indicate that the antigen recognized by the 2F3 MAb is its synthetic product (N-acetyl-t-fucosamine). These conclusions must be correlated with a possible polar effect of the transposon.

Another aspect of our findings is the presence of the antigen recognized by the 2F3 MAb on the O26 O antigen from the EHEC and EPEC strains and not on the O26 O antigen from the non-EHEC and non-EPEC strains. This fact may be explained by a genetic alteration or modification of O-antigen biosynthesis after a lateral gene transfer or a lysogenic conversion followed by recombination (10). Because of the strong association between the eae gene and the antigen recognized by the 2F3 MAb among O26 EHEC and EPEC strains, it is possible that the strain acquired them in the same moment of evolution. Nevertheless, the specificity of the 2F3 MAb for the O26 EPEC and EHEC strains allows the identification of these pathogenic strains within the O26 serogroup. The 2F3 MAb can therefore be considered a pertinent epidemiological tool.

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