A New Adherent Form of an Attaching and Effacing Escherichia coli (eaeA+, bfp−) to the Intestinal Epithelial Cells of Chicks

Masuo SUEYOSHI, Hidehiko FUKUI1, Shogo TANAKA, Munee NAKAZAWA2, and Kenichiro ITO3
Kyushu Branch Laboratory, National Institute of Animal Health, 2702 Chuzan, Kagoshima 891-01, 1Shiga Livestock Hygiene Service Center, 226 Nishihongo, Ohmihachiman, Shiga 523, 2Feed Safety Research Division, National Institute of Animal Health, Tsukuba, Ibaraki 305, and 3Department of Bacteriology, National Institute of Health, Toyama, Shinjuku-ku, Tokyo 162, Japan
(Received 26 April 1996/Accepted 2 July 1996)

ABSTRACT. The adherent site of “attaching and effacing Escherichia coli” (AEEC; O103: H−, SK-1 strain) on the intestinal epithelial cells of chicks infected naturally and experimentally was ultrastructurally investigated. The eaeA gene was detected by polymerase chain reaction in the SK-1 strain of E. coli isolated from the intestinal content of a chick infected naturally, however, the bundle-forming pilus (bfp) gene could not be detected. The SK-1 strain (bfp−) of AEEC could attach to the intestinal epithelial cell and induce attaching-effacing lesions in the intestine of chicks. Transmission electron microscopy revealed numerous pilus-like microfilaments in the space between colibacilli and the membranes of the intestinal epithelial cells. The present study suggests that SK-1 strain (eaeA+, bfp−) may attach closely to the intestinal epithelial cells by a novel adhesin different from bfp. — KEY WORDS: adherence, attaching and effacing E. coli, chicken.


Enteropathogenic Escherichia coli (EPEC) isolated from several animals is one of the diarrheagenic E. coli [1, 4, 11]. Some EPEC attach closely to intestinal epithelial cells, efface the microvilli, and are named attaching and effacing E. coli (AEEC) [11]. AEEC induces the characteristic “attaching-effacing (AE)” lesions [11], and field cases of AEEC infection have been reported in calves [3, 5, 10, 17, 21], goat kids [16], piglets [20], chicks [2, 15] and pigeon [19] in Japan. The mechanism of the production of AE lesions has never been clarified in detail, however, the adherence to the intestinal epithelial cells may be an important virulence factor of the colibacilli. It has been reported that colibacilli adhere to intestinal epithelial cells by virtue of bundle-forming pilus (bfp) bacteria in the early stages of infection [1, 4, 18]. The eaeA gene produces an intimin (94 kDa outer membrane protein) and induces a profound accumulation of actin, myosin, α-actinin, talin, and ezrin beneath the adherent microorganisms, with formation of cuplike pedestals [1]. As the result of these cytoskeleton changes in the intestinal epithelium, the AE lesions develop. We have already reported AE lesions on the intestines of chicks naturally infected with E. coli [2], and AE lesions could also be reproduced in the cecum of chicks experimentally infected with E. coli (SK-1 strain) isolated from chicks [15]. This paper describes the ultrastructure of the attachment site of AEEC (SK-1 strain) to the intestinal epithelial cells of chicks, and the search for a bfp gene in AEEC by the polymerase chain reaction (PCR).

The presence of the virulence factor genes of the SK-1 strain was examined by PCR as follows. The oligonucleotides used for amplification of bfp sequences were the previously described primer system bfps (5'-GAAGTAAAGCGCAAGCTC-3') and bfpas (5'-ACATGCGCGTTATACACC-3') [7]. The eaeK1 (5'-CGCTTATTTCTGTTTATGAT-3') and eaeK4 (5'-TCGCGGTTACAGAGATC-3') were used for amplification of eaeA sequences [12]. Each primer was synthesized on the basis of the published nucleotide sequence of eaeA from E. coli E2348/69 (GenBank accession no. M58154). The oligonucleotides used for amplification of heat-stable toxin (ST), heat-labile toxin (LT) and verotoxin (VT) sequences were the previously described primer system (ExE) [6]. Using 0.1 μM of each primer and 4.8 μl of processed bacterial suspension, PCR was performed under standard conditions (Promega Corp., U.S.A.) [14]. The SK-1 strain was also examined by slide agglutination with specific antisera for the fimbrial adhesin F4 (K88), F5 (K99), F6 (987P) and F41. The specificity of these antisera was reported previously [13].

Pathologically, 6 broiler chicks naturally infected, and 10 chicks experimentally infected with AEEC, were examined by electron microscopy. In the natural cases, specimens obtained from the ileum and the cecum were fixed in 10% buffered formalin, and were postfixed in 1% osmium tetroxide-phosphate buffer. After having been dehydrated through a graded series of ethyl alcohol-water mixtures, and embedded in epoxy resin (Nacarai Tesque, Kyoto), ultrathin sections were cut, stained with uranium acetate and lead citrate, and examined with a transmission electron microscope (JEM-1010, JEOL). In the experimental cases, the samples taken from the cecum were fixed in 2.5% glutaraldehyde-phosphate buffer (pH7.4) at 4°C for 2 hr and washed three times for 15 min each in 0.1 M phosphate buffer. They were postfixed in osmium tetroxide-phosphate buffer at 4°C for 1 hr. The subsequent procedure was the same as that used in the natural cases.

The following results were obtained from the examinations mentioned above. The E. coli had an eaeA gene, but did not possess a bfp gene, ST, LT or VT. The SK-1 strain did not possess any fimbrial adhesins as F4 (K88), F5 (K99), F6 (987P) and F41. Transmission electron microscopic examination of samples obtained from the ileum of chicks infected naturally with AEEC revealed that numerous colibacilli were attached to superficial membranes.
of the intestinal epithelial cells as previously reported [2]. Some cell membranes formed cup-shaped invagination and a pedestal-like protrusion associated with the attached bacteria. Almost all the microvilli of epithelial cells were lost or disoriented, and the associated cytoskeletons were disrupted in the regions of bacterial attachment. A concentration of electron-dense material was also seen beneath the adherent organisms, and some pilus-like substances were confirmed on the surface of the bacteria (Fig. 1). There were also numerous microfilaments in the spaces between bacteria and the intestinal epithelial cells. In the chicks of experimental infection, the typical AE lesions were observed on the cecal mucosa [15]. The structure of the membrane of the epithelial cell was clarified (Fig. 2). The cell wall and cell membrane of the bacteria were also strictly maintained. Delicate and short pili were also confirmed on the surface of bacteria. There were also numerous microfilaments in the space between the bacterial wall and the membrane of the epithelial cell. A few pilus-like substances were confirmed on the luminal surface of colibacilli.

The adherent form of the SK-1 strain was different from that of Enterotoxigenic E. coli (ETEC). ETEC attaches to the microvilli of intestinal epithelial cells by the fimbrial adhesin [9]. However, there was nothing in the space between AECC and the surface of epithelial microvilli in this study. The microvilli were markedly elongated on the first AECC adherent site on the surface of the epithelial cell [15]. Pili-like microfilaments were often confirmed on

Fig. 1. Transmission electron micrograph of the ileal epithelial cell from a naturally infected chick. Colibacilli (C) closely attach to the surface of the epithelial cell (E). Concentration of electron dense materials are seen beneath adherent organisms. There are some pilus-like substances (small arrows) on the bacterial surface. There are also numerous microfilaments (large arrows) in the space between the bacterium and epithelial surface.

Fig. 2. Transmission electron micrograph of the cecal epithelia from a chick experimentally infected with AECC (SK-1 strain, bfp-). The delicate and short pili (small arrows) are confirmed on the bacterial surface. Numerous microfilaments (large arrows) are confirmed in the space between the colibacilli (C) and the epithelial cell (E).
the site of the intimate attachment with AECC, and might be related to the changing of the cytoskeleton. The alteration of the cytoskeleton was associated with the eaeA gene [1, 8], and there is the possibility that the interconnection between the bacteria and intestinal epithelial cell is associated with the eaeA gene.

The space between the bacteria and intestinal epithelial cells was about 20 nm in width in the natural infection, whereas, this space was about 11 nm in the experimentally infected chicks. The difference in this distance is probably dependent on the liquid and the condition of the time and/or the temperature of the fixation.

Although numerous strains of AECC isolated in Thailand were bfp positive, almost all strains of AECC isolated in Japan were bfp negative (unpublished data). We will be investigating further the point of adherence of AECC (bfp-) and AECC (bfp+) to the intestinal epithelial cells by electron microscopy.

It has been reported that bfp was necessary for initial localized adherence [1, 4, 18], however, AECC (SK-1) could attach to intestinal epithelial cells of chicks in this study. This suggests that the SK-1 strain (eaeA+, bfp-) may attach to a novel adhesin different from bfp.

ACKNOWLEDGMENTS. We wish to thank Messrs. K. Kawasaki, T. Fujisawa and Y. Ando for their help in part of this study.

REFERENCES