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Adherence Pili of Avian Strains of *Escherichia coli* O78

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Avian colisepticemia starts with the invasion of the trachea by virulent strains of *Escherichia coli*, with strains of serotype O78 responsible for about 50% of the cases. Several O78 strains isolated from poultry with colisepticemia produced pili with a subunit of molecular weight of 18,000 that mediated adherence to avian epithelial tissues in vitro and in vivo.

In poultry, infection with virulent strains of *Escherichia coli* starts in the upper respiratory tract and develops as the bacteria invade the air sac system and vital organs. The resulting septicemia involves high morbidity and mortality.

One of the virulent *E. coli* strains frequently associated with avian colisepticemia belongs to serotype O78 and was previously shown to contain a variety of adherence pili—colonization factor antigen I (CFA/I) (4, 5), CS31A (K88-related pili) (6), and *pap* pili (T. Korhonen, personal communication). We examined the possibility that avian O78 bacteria also produce pili that facilitate adherence to the avian trachea. Such pili could indeed be observed by electron microscopy (1, 3, 9, 10), and their role in pathogenesis has been demonstrated (10). The results presented in this communication indicate that these pili, named AC/I (avian *E. coli* I), have subunits with an apparent molecular weight of about 18,000. Although it was not possible to show hemagglutination of erythrocytes (RBC), we did show that the pili are important for adherence to avian epithelial cells both in vitro and in vivo. Furthermore, we demonstrated that adherence can be inhibited by monoclonal antibodies directed against the protein subunit of the pili.

Strains of *E. coli* that are virulent for poultry were isolated from chickens or turkeys with acute colisepticemia. They were serotyped and studied for the production of pili. Figure 1 represents a typical cell of avian *E. coli* O78 bacteria covered with thin pili. Piliated avian O78 bacteria were similar in appearance to human O78 bacteria covered with CFA/I pili. However, in contrast to the CFA/I pili, which agglutinate RBC from humans and calves, the pili from avian strains did not agglutinate these two types of RBC. In addition, the pili of O78 avian bacteria did not react in an enzyme-linked immunosorbent assay (ELISA) (14) with four types of monoclonal antibodies against CFA/I (kindly provided by W. Paranchych) (15). Additional tests indicated that the piliated avian O78 bacteria failed to agglutinate RBC from chickens, turkeys, rabbits, sheep, horses, mice, guinea pigs, and humans. The piliated bacteria were also negative in agglutinating beads coated with α-D-Gal-(1-4)-β-D-Gal disaccharides (Orion Diagnostica, Espo, Finland) recognized by the P pili (2).

Pili were prepared as described by Salit and Gotschlich (11) from cultures grown in Minca agar plates (7) for 48 h at 37°C. The molecular weight of the subunit of the pili was determined in polyacrylamide gel electrophoresis and found to be about 18,000 (Fig. 2). We named the pili AC/I.

To further study the AC/I pili and their function, monoclonal antibodies were prepared as described by Kohler and Milstein (8). Mice were immunized with formaldehyde-fixed cells, and the resulting hybridoma supernatants were screened against a pilus preparation by use of the ELISA method. Five different clones were selected for examination. All of them agglutinated piliated bacteria as well as purified pili. One clone, 875, produced antibodies that reacted with the protein subunit of the pili (Fig. 2). None of the antibodies reacted with bacteria of O78 strains carrying pili of the K99, K88, CFA/I, CFA/II, or *pap* types or with the corresponding purified pilus preparation.

The role of AC/I pili in adherence to tissues was studied by using an ELISA reaction in the following way. Segments of tracheas or intestines from 14-day-old chickens were cut longitudinally, washed several times with phosphate-buffered saline, and scraped into phosphate-buffered saline to give an epithelial cell suspension (about 5×10^7 cells per ml). A 50-μl volume of the suspension was put into each of 96 U-shaped wells of a microdilution plate. The plates were centrifuged for 10 min at 500 × *g* and the supernatant was removed. The cells were incubated with 50 μl of a washed bacterial suspension (about 2×10^9/ml) after being blocked with 200 μl of 0.5% gelatin. The fraction of bacteria that adhered to the epithelial cells was determined by ELISA as described by Voller et al. (14) with anti-O78 rabbit serum and alkaline phosphatase-conjugated goat anti-rabbit serum. The results indicated that piliated O78 bacteria of avian isolates adhered much better than unpiliated bacteria. Furthermore, if the bacteria were grown at 18°C, a temperature at which the synthesis of pili is inhibited (10), adherence was appreciably lower (Fig. 3). It should be noted that the bacteria adhered to intestinal epithelial cells as well as to tracheal cells. This finding may indicate that the virulent bacteria can be carried and shed through the alimentary tract of chickens. Carrier chickens show no signs of disease, because the bacteria do not produce enterotoxin and are not virulent when given orally (M. W. Naveh and E. Z. Ron, unpublished data).

Additional evidence for the role of AC/I pili in adherence to avian trachea was obtained from the data presented in Fig. 4. The results indicate that the adherence of cells producing AC/I pili to epithelial cells from avian trachea could be blocked by the addition of the monoclonal antibodies that react with the pilus subunit in an immunoblot assay. As a control, the same experiment was performed using the monoclonal antibodies against CFA/I pili, which had no inhibitory effect on the adherence of AC/I-producing cells to avian epithelium.

To provide evidence for the in vivo role of AC/I pili, we

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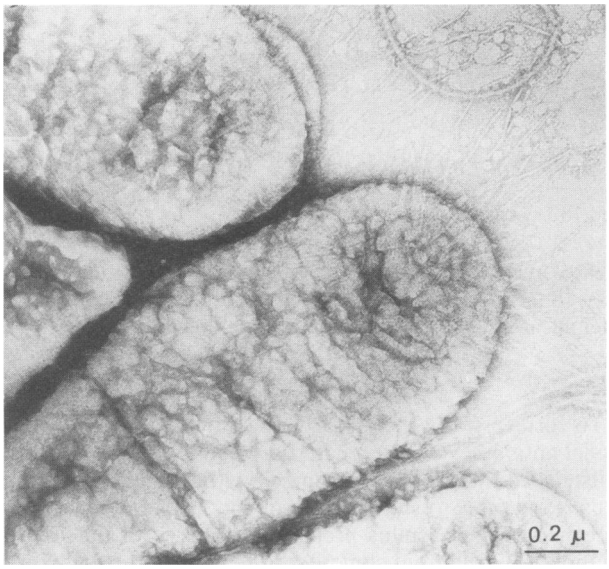

FIG. 1. Piliated cells of avian *E. coli* O78 bacteria. Cells of *E. coli* O78 isolated from avian colisepticemia (strain 784) were prepared and stained as described previously (10). They were examined in a Jeol 100B electron microscope at 100 V. Magnification, ×28,700.

administered 0.2-ml volumes of the suspension containing 10^6 cells of *E. coli* O78 intratracheally into 3-week-old chickens. Six hours later, the tracheas were aseptically removed and suspended in sterile saline, and the number of adhering *E. coli* cells was determined by viable count. The results indicated that piliated bacteria (isolate 781) were much more effective in adhering to the trachea than the same bacteria grown at 18°C to inhibit pilus production (Fig. 5). No *E. coli* cells could be detected in the tracheas of untreated chickens.

To find out whether AC/I pili mediate preferential binding to avian tissues, we examined the adherence of avian strain

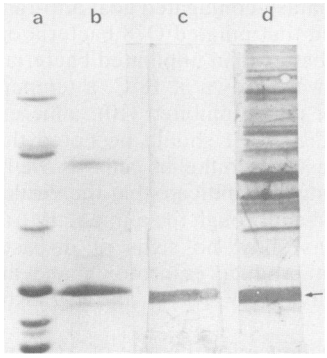

FIG. 2. Electrophoresis and immunoblots of AC/I pilus preparations. Electrophoresis was carried out in 18% · polyacrylamide–0.009% bisacrylamide by the method of Thomas and Kornberg (12). Gels were either stained for proteins with Coomassie brilliant blue R-250 (lanes a and b) or subjected to immunoblotting on nitrocellulose membranes (lanes c and d) as described previously (13). Lanes: a, molecular weight standards (from bottom to top, 12,300, 14,300, 18,400, 24,000, 43,000, 66,000); b, AC/I pilus preparation from strain 781; d, polyclonal anti-O78 mouse serum; c, ascitic fluid containing monoclonal antibody 875. The arrow indicates the position of the 18-kilodalton protein band.

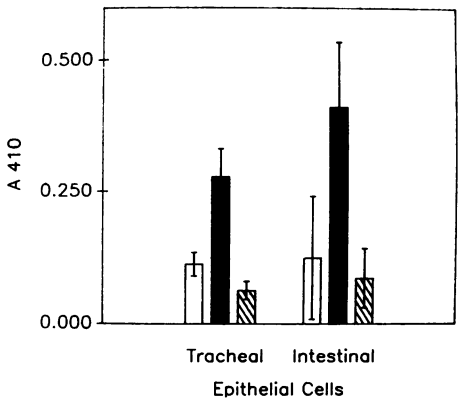

FIG. 3. Adherence of bacteria to avian epithelial cells. The preparation of avian tracheal epithelial cell suspension (5×10^7 cells per ml) and bacterial suspension (2×10^9 cells per ml) is described in the text. The adhering bacteria were quantified with anti-O78 rabbit serum and alkaline phosphatase-conjugated goat anti-rabbit antiserum by ELISA as described by Voller et al. (14). An A_{410} of 0.5 represents about 10 bacteria per epithelial cell. Bars indicate adherence of cells of strain 781 grown at 18°C (not expressing AC/I pili) (□), of strain 781 grown at 37°C (expressing AC/I pili) (■), and of unpiliated strain 278 grown at 37°C (▨).

781 to avian tracheal epithelial cells and compared it with adherence to human buccal cells. The experiment was performed as described for Fig. 3. The buccal cells were obtained by scraping and suspending them in phosphate-buffered saline and were treated in the same way as the avian cells. The results indicated that the bacteria carrying AC/I pili adhere preferentially to avian epithelium (Fig. 6). These results suggest the existence of species specificity, a conclusion strengthened by the finding (Fig. 6) that the effectiveness of binding is reversed in a human isolate of the same serotype (strain H10407; kindly provided by K. Jann) carrying CFA/I pili.

This paper presents experiments designed to characterize the adherence pili of pathogenic *E. coli* strains of serotype

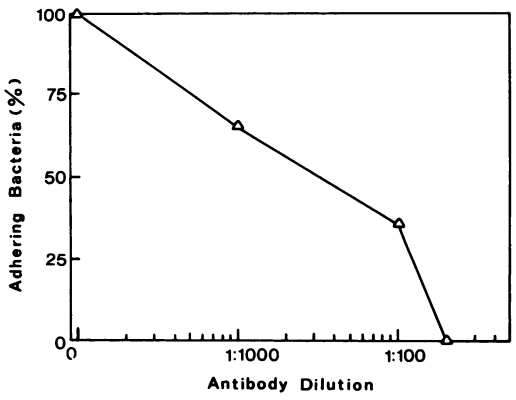

FIG. 4. Effect of anti-pilus monoclonal antibodies on adherence of piliated bacteria to avian epithelial cells. Piliated cells of strain 781 (10^9) were incubated for 10 min with increasing concentrations of monoclonal antibody 875 and then were incubated with tracheal epithelial cells and treated as described in the legend to Fig. 3. The 100% value was calculated by determining the A_{410} as for Fig. 3 and subtracting from this value the value obtained with the controls, which involved incubation of the piliated cells with nonspecific anti-CFA/I antibody.

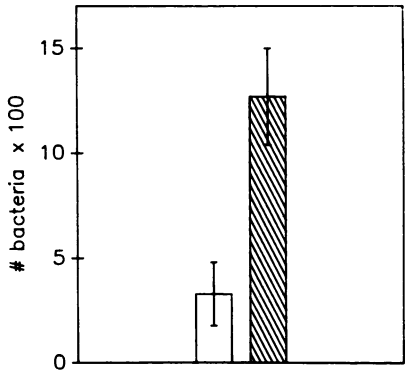

FIG. 5. Adherence of bacteria to chicken tracheas. Chickens at 21 days old were given 0.2 ml of inoculum containing 10^6 cells of *E. coli* O78 intratracheally. Six hours later, the tracheas were aseptically removed and suspended in sterile saline, and the number of adhering *E. coli* cells was determined by viable count. Avian strain 781 was grown at 18°C (AC/I pili not expressed) (□) and at 37°C (expressing AC/I pili) (▨).

O78 isolated from poultry with colisepticemia. Although avian colisepticemia is economically important, the bacteria involved in it have not been extensively studied. The pili of these bacteria are of special interest because they are probably the factor responsible for bacterial recognition of avian tissues. Of additional interest is the fact that the avian disease is presumably initiated via the trachea, a situation that is not common in *E. coli* diseases of humans and other mammals. The AC/I pili described in this paper were found in about 50% of the avian isolates of strain O78. Their role in mediating adherence to avian epithelial cells could be shown in vivo as well as in vitro. This adherence appears to be avian specific, as the bacteria bind better to avian tissues than to human buccal epithelial cells. This specificity makes them a good model system for studying bacterium-host recognition and interaction in poultry.

The ability to adhere to the trachea is probably an essential property of avian *E. coli* pathogens. The relationship of these pathogens to the avian host is, in many ways, similar to that of human strains that cause urinary tract infection; that is, to cause disease the pathogens must be able to attach to an epithelial surface that does not allow massive bacterial colonization. After attachment, there is a local infection (human cystitis and pyelonephritis or avian tracheitis and air sacculitis) that either can be overcome at this stage or can develop into bacteremia and sepsis. This is a very different situation from the one in which cells adhere to the intestinal tract, and it remains to be seen if this difference is in any way reflected in the structure of the adherence pili.

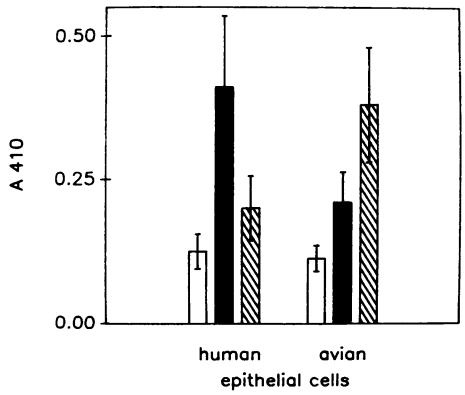

FIG. 6. Adherence of bacteria to avian and human epithelial cells. The experiment was carried out as described for Fig. 3. Buccal cells were prepared by scraping and then by the same washing and treatments performed for the tracheal epithelial cell suspension. Bars indicate adherence of unpiliated strain 278 (□), of strain H10407 expressing CFA/I pili (■), and of strain 781 expressing AC/I pili (▨).

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