Hemagglutination by Escherichia coli in Septicemia and Urinary Tract Infections

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The agglutination of erythrocytes from various animal species by Escherichia coli was studied. The 405 strains of E. coli were isolated from urine in patients with urinary tract infections, from blood in septicemic patients, or from feces in persons without intestinal or urinary disorders. In urinary tract infections, D-mannose-resistant agglutination (MRHA) of human erythrocytes was the most common finding (23% of the strains). The highest frequency of mannose-sensitive hemagglutination (MSHA) attributed to type I (common type) pilu occurred with guinea pig erythrocytes (11.5%). Of the 78 E. coli strains isolated from blood cultures, 11 (14%) produced MRHA of human erythrocytes and only one gave MSHA. In the stool cultures, only 1 of 170 E. coli strains was MSHA reacting, whereas 28 strains (16.5%) showed MRHA of human erythrocytes. No MRHA strain reacted with antiserum against colonization factor antigen (CFA)/I of pilus nature in enterotoxigenic human E. coli strains (O78:H12). MRHA of bovine erythrocytes, reputedly typical of enterotoxigenic E. coli of serogroups O6 and O8, was shown by only two strains, neither of which agglutinated with CFA/II antiserum. The most common hemagglutinating pattern of E. coli from urine and blood thus was MRHA for human erythrocytes. This agglutination may have been caused by pili or other surface properties of one or more serotypes. These may represent a new class of colonization-promoting antigens (adhesins).

In 1921 Dugeon et al. (2) suggested that hemolytic activity could be important for the virulence of Escherichia coli in the urinary tract. Later studies confirmed a high incidence of hemolytic E. coli strains in urine from patients with urinary tract infections. Recently, Minshew et al. (14) found that 49% of E. coli strains from such sources were hemolytic, but only a very few in stool cultures from controls were. These writers also reported a relationship between production of colicin V and virulence for chicken embryos. They extended a preliminary study by Powell and Finkelstein (19), who had found a relationship between agglutination (HA) of chick erythrocytes and virulence in experimental infections.

High correlation has been observed between HA of erythrocytes from different species caused by pili and production of enterotoxin by porcine, bovine, and human strains of E. coli (24). Colonization-promoting antigens with hemagglutinating properties of pilus nature have been characterized in human, porcine, and bovine strains of enterotoxigenic E. coli (7–10, 13, 15, 23). More recent studies demonstrated colonization factor antigen (CFA)/I and CFA/II in human enterotoxigenic strains of the O6, O8, and O78 serogroups (nomenclature of Evans and Evans [7] and Smyth et al. [23]).

Morphologically, all these antigens were shown to be of pilus or pilus-filament type and to agglutinate erythrocytes in a mannose-resistant fashion (MRHA). Type I (common type) pilus, on the other hand, and a recently described surface antigen (6) cause HA which is inhibited by mannose (MSHA). These latter factors may also be important for the colonization of eucaryotic cells (6, 16, 20, 26).

In stool cultures from healthy Swedish and Ethiopian subjects (25), we seldom found enterotoxigenic E. coli strains that produced CFA, although we isolated strains which caused MRHA for human erythrocytes, as does CFA/I (23). The same strains, however, did not react with CFA-specific antiserum. These observations prompted us to study human E. coli cultured from feces, urine, and blood to determine the relative frequencies of HA by strains from the various sources.

MATERIALS AND METHODS

Bacteria. Altogether, 405 strains of E. coli were used in the tests. Blood cultures yielded 78 strains,
and acute urinary infections yielded 157 strains. These were collected over a 1-year period from patients in the departments of medicine, surgery, and pediatrics at the Karolinska Hospital. The 170 control fecal strains were isolated from 41 patients in the same hospital. These were free from diarrhea and urinary tract infection. All strains were stored on deep agar at +4°C and in glycerol broth at −70°C.

Cultures. Each E. coli strain was cultured on CFA agar (8) and in nutrient broth in nonaerated cultures for 24 h at 37°C. Some strains which showed typical HA of the various patterns were also cultured on conventional diagnostic media—Endo-agar, McConkey, CLED, DC, and blood (horse blood) agars. The ingredients for the media were purchased from Difco (Detroit, Mich.).

HA. Citrated blood (human, bovine, chick, and guinea pig) was washed twice in 0.15 M NaCl and suspended to 5% (wt/vol) (29). Screening of strains was performed in round-bottomed plastic Microtiter plates (Flow Laboratories, Irvine, Scotland). Each positive strain was recultured and restested for HA of erythrocytes from the various species on glass depression slides as used in “hanging-drop” motility tests. Equal volumes of bacterial suspension (2 × 10^9 to 4 × 10^9 bacteria/ml) and erythrocyte suspension were mixed.

In tests for MRHA, 1 drop of bacterial suspension, 1 drop of 0.1 M D-mannose in 0.15 M NaCl, and 1 drop of erythrocyte suspension were mixed. The mixture was incubated at 20 and at 4°C for 1 h.

SeroLOGY and other tests. Antisera to CFA/1 and CFA/II antigens were produced in rabbits, and immunoglobulins were purified and stored as recently described (23). The reactivity of E. coli isolated with CFA/1 and CFA/II was tested by slide agglutination. For heat-labile enterotoxin the Chinese hamster ovary (CHO) cell test (11) was used. Each microbial isolate was typed to species level with the API 20E test system according to the manufacturer’s instructions (API System S.A., La Balme les Grottes, France).

RESULTS

Of the 405 E. coli strains, 147 (36%) showed positive HA with erythrocytes from one or more species after culture on CFA agar. Of the 157 E. coli strains from urine in urinary tract infections (1 strain per patient), 28 (17.8%) showed MSHA and 69 showed (43.9%) MRHA, i.e., positive HA reactions in a total of 97 patients (62%). In 16 (20%) of the 78 strains isolated from blood in septicemic patients, the HA test was positive. Of the 170 control fecal strains of E. coli, 96 (21%) gave a positive HA reaction.

The HA profile of the erythrocytes from the various species is presented in Table 1. The results from parallel tests on glass slides with D-mannose added to discriminate MSHA are also shown. Positive MRHA reactions were more common than MSHA with E. coli strains from all three sources, and human erythrocytes were preferentially agglutinated. No difference was detectable between the HA patterns of human erythrocytes from group A and group O blood. E. coli strains which agglutinated chick and bovine erythrocytes were rare in all three sample types.

Table 2 shows the remarkable finding that MSHA reactions of guinea-pig erythrocytes were relatively common with E. coli from urinary tract infections (11.5%) but were extremely rare with strains from septicemic blood or from feces.

Comparisons of cultures on various solid media showed CFA agar to be a reliable standard medium for expressing the HA reactions listed in Table 1. Conventional media, with the possible exception of blood agar, were less satisfactory, and Endo and CLED agar seemed effectively to inhibit expression of HA. Aerated liquid cultures appeared to inhibit strains giving all the HA patterns listed in Table 1 (one strain of each type was tested). Prolonged culture in tubes with liquid medium and a small surface area, on the other hand, enhanced HA expression in weakly positive strains grown under standard conditions. Pellicle formation, as observed in classical studies on piliated E. coli (3–5), was not a common finding.

All the strains that showed MRHA for human and bovine erythrocytes were tested for agglutination with specific CFA/1 and CFA/II antisera. All the tests were negative. The same strains were also tested for heat-labile enterotoxin by the CHO test. All of these tests were negative.

DISCUSSION

Although more than two decades have elapsed since HA and piliation were first reported to be common surface properties of E. coli and other enteric microorganisms isolated from patients with infections, these properties were only recently suggested as important factors in virulence (13). In the past few years, however, it has been clearly shown that piliated E. coli attach more readily to human buccal and uroepithelial cells than do nonpiliated strains (26). Extensive studies on binding of E. coli strains with type 1 pilus—strains which cause MSHA in guinea pig erythrocytes—to monkey kidney cells support the concept of a lectin-like interaction between bacterium and the eucaryotic cell surface (20). However, the recent discovery (6) of a new surface component of nonpiliated nature in urinary E. coli strains with similar lectin-like properties indicates that the interaction of various surface components with eucaryotic cells may be much more complex than is generally expected. Such complexity was indicated already in the early
studies by Duguid (3). Manifold combinations of the various surface components causing HA may thus complicate the interpretation of HA profiles as presented in Table 1.

That other pilus structures do not cause HA should be kept in mind, and also that the Adh factor causes adhesion of certain enteropathogenic E. coli to the epithelial surface of the small intestine (28). Although early work showed that certain culture conditions were critical for pilus and pellicle formation, and although this was confirmed in recent studies (18), the available literature contains no systematic analysis of the influence of different culture conditions on the production of pili and pellicles. From the studies on CFA/I and CFA/II, however, it is clear that only certain culture media permit the expression of these surface antigens. We found that culture of strains with different HA profiles on conventional media, except for blood agar, effectively prevented the expression of all types of HA reaction. In a recent study (12) on hemagglutinating strains of Staphylococcus saprophyticus, HA was expressed in liquid cultures but not on blood agar.

The recent reports (7,8) that human enterotoxigenic strains of E. coli cause MRHA preferentially of human erythrocytes (serogroup O6 and O8 with CFA/II) encouraged us to investigate the incidence of these colonization factors in various infections when CFA antisera became available (23). It seems remarkable that, although numerous E. coli strains showing MRHA reactions were isolated from all three sources, not a single strain agglutinated with the CFA/I and CFA/II antisera. From studies on a small number of CFA/I and CFA/II strains (7,8, 23), the suggestion was advanced that these pilus antigens are controlled by plasmid genes in a fashion similar to the regulation of K88 and K99 in enterotoxigenic E. coli strains of porcine and bovine origin. To our knowledge, however, no systematic study on the epidemiology of these CFA antigens in strains of various origins has yet been reported.

Recent observations on the complexity of various antigenic types of pili in Neisseria gonorrhoeae, with differing degrees of immunological cross-reaction (1), indicate that the picture may be fairly complicated also with coliform organisms. Although the pili of coliforms have been closely studied, little attempt has been made to classify them as in a serotyping scheme. E. coli strains from the urinary tract were observed by Duguid and Campbell (4) in early studies to cause MRHA of human erythrocytes. But it was only recently that a survey of 142 E. coli strains from extraintestinal infections confirmed a high incidence of this type of HA reaction (14).

Ample evidence supports the hypothesis that K88, K99, and CFA/I, all of which give MRHA reactions, are important factors in the colonization of the small intestine as the first stage of enterotoxigenic E. coli diarrhea in animals and humans (7, 8, 13). Recent observations in our laboratories also suggest that a variety of pili and pilus-like structures, such as K88 and K99, reduce the surface charge and enhance the hydrophobic surface properties of these strains (22,
27). These physicochemical phenomena promote the aggregation of bacterial cells, and in all probability also that of bacteria, with epithelial cell surfaces (22, 27).

Studies on urinary tract isolates of MSHA-producing E. coli (16, 20, 26) have shown that type I pilus probably is an important colonization factor (adhesins) in infections of the urinary tract. In our investigation the high incidence of MRHA reactions by E. coli from the urinary tract seems likewise to indicate their colonizing significance, possibly also on other mucosal surfaces. Preliminary studies on one strain of this type (G. Kallenius and R. Möllby, submitted for publication) showed good adherence to periurethral cells. The incidence of MRHA reactions by E. coli from feces was higher in our study than in earlier reports (3–5). The possibility of feces as a reservoir of such strains seems worthy of note.

One may be tempted to speculate that the number of hemagglutinating E. coli strains in blood from septicemic patients reflects the ability of these strains to colonize the urinary tract or other mucosal surfaces, such as the respiratory tract or the intestine. In further studies we hope to elucidate the specificity of hemagglutinating strains of the groups listed in Table 1 to adhere to human epithelial cells from various sites. Encouragement for further studies on additional virulence factors, including cytotoxic (hemolytic) properties in this collection of strains, is provided by the recent observation of high virulence for chicken embryos and colicin V in human (14) and calf (21) strains. Recently developed pili vaccines with high prophylactic efficacy against porcine and calf diarrhea, and probably also against enterotoxigenic E. coli diarrhea in humans (15), will undoubtedly also prompt further epidemiological studies on the frequency of HA and other virulence factors in E. coli from intestinal and extraintestinal infections in humans and animals.

However, the possible role of other bacterial surface components, such as capsule carbohydrates or proteins, as colonizing factors in the first stage of an infection, also requires additional study in view of the recent findings on special enteropathogenic strains of E. coli (18) and Bacteroides fragilis (17).

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LITERATURE CITED


