Review article

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Esch. coli infections in childhood*

Significance of bacterial virulence and immune defence

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The first encounter of the neonate with *Esch. coli* consists of the normal intestinal colonization. Already at this stage the body is able to cope with these micro-organisms, accepting them as a part of the normal intestinal flora, but hindering them from invading the tissues. It is obvious, however, that from this early colonization onward the infant and then the child is carrying a reservoir of potentially pathogenic micro-organisms which are separated from the tissues only by the mucous membranes, supported by the host defence. This paper discusses *Esch. coli* infections in childhood with special reference to bacterial virulence factors and the immune defence mechanisms involved.

Normal colonization of the neonate

Soon after birth the infant is colonized by bacteria, and among other species *Esch. coli* appear in the gut (Hanson and Winberg, 1972; Gothefors *et al.*, 1976). Only in some neonates are these *Esch. coli* of the same serotype as the dominant strain in the mother's faecal flora. The origin of the remainder is not clear but hospital flora may be one source. In some cases it has been noted that a baby born in hospital first picks up an *Esch. coli* strain which only later is also found in the maternal stool (Gothefors, 1975; Gothefors *et al.*, 1976). Such colonization normally induces an antibody response against the *Esch. coli* at the serum and possibly local level, as shown by Lodinová, Jonja, and Wagner (1973) using an *Esch. coli* O83 for experimental colonization of infants.

What are the factors which normally prevent these potential pathogens from invading the neonate from the gut? One poorly defined, but yet probably very important, mechanism is the 'colonization resistance' especially provided by the normal anaerobic flora of the gut (van der Waaij and Heidt, 1976).

Probably the baby is also colonized early with these micro-organisms which are not well defined.

Another significant mechanism is the immune defence in mucous membranes, including those of the gut. Here a major component consists of locally produced secretory IgA (SIgA) antibodies. Such antibodies can prevent infection primarily by the simple function of binding micro-organisms, thereby hindering their contact with the mucous membranes and underlying tissues. During the first week of life the local antibody production in the form of SIgA may be present but sparse—SIgA consistently appears in the saliva at a few weeks of age (South, 1971). At this age such antibodies are normally provided by the maternal milk which contains large amounts, SIgA making up almost half the protein content of the early colostrum (Hanson 1961; Hanson *et al.*, 1971). As the SIgA levels rapidly diminish in the colostrum within the first few days after birth, there is a simultaneous increase in the milk volume which compensates for this decrease in antibody concentration (Schubert and Grünberg, 1949; Carlsson *et al.*, 1976b) (Fig.), so that there is a high and rather constant output of SIgA antibodies throughout lactation.

It is striking that human milk contains SIgA antibodies against a multitude of O and K antigens of *Esch. coli* (Gindrat *et al.*, 1972; Carlsson *et al.*, 1976b; Hanson *et al.*, 1975b, 1976a)—surprising considering that the milk SIgA obviously is synthesized in the mammary gland (Hanson *et al.*, 1975b) and it is difficult to conceive how these enterobacterial antigens can induce local antibody production in the mammary gland. Recently it was possible to study this, however, on the basis of the observation that human milk contains large numbers of lymphoid cells (Smith and Goldman, 1968; Murillo and Goldman, 1970; Ahlstedt *et al.*, 1975a). Almost half of these are B lymphocytes (Diaz-Jouanen and Williams, 1974), primarily producing IgA antibodies (Smith and Goldman, 1968; Murillo and

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FIG.—Schematic representation of the volume of human milk and its content of secretory IgA antibodies to *Esch. coli* O antigens after parturition. The decrease in antibody concentration is compensated by the increase in milk volume (cf. Carlsson *et al.*, 1976b).

Goldman, 1970; Ahlstedt *et al.*, 1975a). Since lymphoid cells from the Peyer's patches of the gut have the capacity to repopulate the gut mucosa with IgA-producing cells in irradiated animals (Craig and Cebra, 1971) it seemed possible that such cells, antigenically triggered in the gut, might home to other tissues rich in lymphoid cells producing IgA. In accordance with such a hypothesis we found that three pregnant women within a few days after intestinal colonization with *Esch. coli* O83 had large numbers of lymphoid cells in their milk, producing SIgA antibodies to the O83 antigen (Goldblum *et al.*, 1975). There was no simultaneous serum antibody response in these women, which speaks against transport of antigens from the gut to the mammary gland and favours the assumption of a homing of antigen-triggered lymphoid cells from the Peyer's patches to the mammary gland. In this way the mother is immunized both locally in the gut and in the milk against the bacteria in her intestine (Ahlstedt *et al.*, 1976). Her breast-fed baby which may be exposed to these bacteria is at the same time provided with milk SIgA antibodies against them. The fact that Swedish and Pakistani mothers have milk antibodies against the same *Esch. coli* O antigens suggests that the broad spectrum of milk antibodies reflects a series of early and recent encounters with prevalent strains of enterobacteria (Carlsson *et al.*, 1976a). As a consequence the milk may provide protection against many Gram-negative bacteria, not only those actually present in the maternal gut flora.

It is not yet definitely proven that the defence

factors of human milk, especially SIgA antibodies, are important for the protection of the infant. Evidence has been presented, however (Hanson and Winberg, 1972; Goldman and Smith, 1973; Gerrard, 1974), and will be further reviewed in relation to various *Esch. coli* infections discussed below.

Esch. coli infections in infancy and childhood

Neonatal sepsis and meningitis. *Esch. coli* is a major pathogen in neonatal sepsis/meningitis. The causative *Esch. coli* often belong to common O groups (Bergström *et al.*, 1972) and are generally assumed to originate from the gut flora. What makes them invade the intestinal mucosa and reach the blood stream and meninges causing such serious disease is yet unknown. It has been noted, however, that the capsular K1 antigen is exceedingly common on *Esch. coli* causing neonatal meningitis, being found in about 80% of strains from infants with this disease (Robbins *et al.*, 1974).

With the K1 antigen presumably being an important virulence factor in these infections it is of some interest that colonization with K1 containing *Esch. coli* induces a serum antibody response only detectable in babies of over 6 months (McCracken *et al.*, 1976; Glode *et al.*, 1976). On the other hand, most of the mothers have serum IgG and IgM anti-K1 antibodies, and about 80% of cord sera contain maternal IgG anti-K1, while absence of the IgG anti-K1 does not relate to increased risk for infection (McCracken *et al.*, 1976). Serum IgA anti-K1 are not so often found in the mothers though milk samples with very few exceptions contain secretory IgA anti-K1. Deficiency in milk anti-K1 may increase the risk of the baby to attract neonatal *Esch. coli* infection, and Winberg and Wessner (1971) showed that a series of infants with neonatal sepsis/meningitis had consumed significantly smaller amounts of human milk than matched controls.

In support of the protective functions of human milk are also the lower number of *Esch. coli* in the stool of breast-fed compared to artificially-fed babies (Bullen and Willis, 1971; Michael, Ringback, and Hottenstein, 1972). This would diminish the infection dose in breast-fed infants and increase their chance of coping with the bacterial assault.

Besides the anti-K1, milk contains antibodies to several other *Esch. coli* K antigens and also many O antigens (Gindrat *et al.*, 1972; Hanson *et al.*, 1976a). It is suggested that all these SIgA antibodies as well as other factors in the maternal milk can play an important role in the control of intestinal flora, preventing neonatal sepsis/meningitis. This de-

fence may be of special significance during the early part of life, till the infant can mount its own local immunity, including the local production of intestinal SIgA antibodies.

Diarrhoeal disease. The enteropathogenic *Esch. coli* may cause severe disease in the infant and small child. The pathogenic mechanisms of these infections are still unclear. Recent studies have suggested that *Esch. coli* may induce diarrhoea in two ways. One involves bacteria which have the capacity to adhere to gut epithelial cells (McNeish *et al.*, 1975) and invade the tissues more or less deeply like *Shigella* and *Salmonella* (Guerrant *et al.*, 1975). The other, where *Esch. coli* produce enterotoxins similar to *Vibrio cholerae* toxin in function (Guerrant *et al.*, 1975; Holmgren and Svennerholm, 1976).

We know little about the bacterial adhesion mechanism. It is possible that bacterial pili are important and that specific protein structures like the K88 present on *Esch. coli* enteropathogenic for the pig are present on *Esch. coli* with enteropathogenic activity in humans. If so, this is of importance, since antibodies to such structures crucial for pathogenicity may be efficiently protective and can perhaps be induced by vaccination.

Our knowledge of enterotoxigenic *Esch. coli* is scanty but the mechanism of their pathogenicity is most probably similar to that of *V. cholerae* with the toxin attaching to specific epithelial cell receptors (Holmgren and Svennerholm, 1976). Secretory IgA antibodies against *V. cholerae* can prevent its adhesion to gut epithelium and its toxigenic effects in experimental models (Fubara and Freter, 1973; Holmgren and Svennerholm, 1976; Pierce and Sack, 1976). This preventive effect is potentiated by the presence of normal gut flora (Schedlovsky and Freter, 1974), probably exemplifying synergism between 'colonization resistance' and immunological factors (van der Waaij and Heidt, 1976).

The lack of, or slow development of local immune defence in the neonate should increase the risk of diarrhoeal disease early in life. This is strikingly illustrated in developing countries where such diarrhoeal infections, especially accompanying undernutrition, often are life threatening. Mata and Urrutia (1971) and Mata and Wyatt (1971) have shown that the risk of such infections increases on weaning. This suggests that breast-feeding may provide protection. This is supported by the notion that infections with enteropathogenic *Esch. coli* do not occur in breast-fed infants (Crosse, 1966) and that epidemics with such bacteria can

be controlled with human milk (Svirsky-Gross, 1958; Tassovatz and Kotsitch, 1961; Largaia *et al.*, 1974).

Recently we studied a small *Esch. coli* O111 outbreak in Oslo (Andersen *et al.*, 1976); 6 of the infants with the infection had severe diarrhoea and had not been breast fed. 2 breast-fed infants had *Esch. coli* O111 in the stool but had only insignificant symptoms. There was no detectable anti-O111 in the milk from these mothers, however. This suggests firstly that even with a lack of specific antibodies to the *Esch. coli* the many other defence factors of the milk (Bullen and Willis, 1971; Bullen, Rogers, and Leigh, 1972; Hanson and Winberg, 1972; Gothefors, 1975) may help to alleviate the infection. Secondly, the infant may be colonized when the milk is antibody deficient. This may not happen when the baby is breast fed by a mother exposed more often to enteropathogenic *Esch. coli*. In accordance with this, the level of milk SIgA antibodies against the O antigens of enteropathogenic *Esch. coli* is much higher in the milk of Pakistani women than Swedish women, the latter supposedly rarely exposed to such *Esch. coli* (Ahlstedt *et al.*, 1976). This was so in spite of the fact that the Pakistani women were severely undernourished, supporting the value of breast feeding also under such circumstances (Carlsson *et al.*, 1976a).

Since SIgA antibodies can help in preventing experimental cholera (Fubara and Freter, 1973; Holmgren and Svennerholm, 1976; Pierce and Sack, 1976), it is of interest that we recently found SIgA antibodies against *Esch. coli* enterotoxins in milk from Pakistani but not from Swedish mothers (Ahlstedt *et al.*, 1976; Holmgren *et al.*, 1976). This suggests that in areas where enterotoxin-producing *Esch. coli* occur, human milk may be important for protection against infantile diarrhoea caused by *Esch. coli*. Direct evidence for this is still lacking however, but it seems reasonable to promote breast feeding in paediatric practice as a preventive measure. In addition it may be possible to develop vaccination programmes for women resulting in improved protection also of their breast-fed babies.

Urinary tract infections (UTI). The most common *Esch. coli* infections in childhood are without doubt UTI. The neonatal infections may appear with insignificant or uncharacteristic symptoms like poor weight gain. During this period the UTI is often accompanied by bacteraemia and is assumed to have a haematogenous route from the gut to the urinary tract. The same bacterial strain is usually found in the gut as in the urine. After

the neonatal period there are usually symptoms accompanying acute cystitis and pyelonephritis, but during the subsequent prevalent recurrences the infections more and more often cause none or only minor symptoms (Bergström *et al.*, 1968).

UTI during early life is related to a significant risk of developing renal parenchymal reduction and scarring (Hodson and Wilson, 1965). Such changes have been seen in 4.5% of girls and 13% of boys followed from their first-recognized infection (Winberg *et al.*, 1974). About one-quarter of these children developed lowered glomerular filtration capacity after an observation period of 8–15 years (Peterson *et al.*, 1976) (Table I).

TABLE I

Renal function in 20 girls with renal scarring after nonobstructive urinary tract infections. Observation time from first noted infection was 8–15 years

Glomerular filtration rate (⁵¹ Cr-EDTA)*	Age at first known infection	
	<1 year	>1 year
>110	7	5
90–110	0	2
<90	2	4

For details see Peterson *et al.*, 1976.

*ml/min per 1.73 m².

It is not clear how the symptomatic forms of UTI relate to the 'asymptomatic' cases of UTI often found on screening schoolgirls and others (Kunin, Deutscher, and Paquin, 1964). Several of the patients with asymptomatic bacteriuria have a history of earlier UTI and in addition 10–26% of them have renal scarring suggesting earlier infections (Kunin *et al.*, 1964; Savage *et al.*, 1973; McLachlan *et al.*, 1975; Lindberg *et al.*, 1975b).

Why do such infections occur and appear in these different forms? Is the host defence abnormal in the urinary tract of some individuals or are other factors like reflux or residual urine mainly to blame? The answers to these questions are not yet available but are of great importance because of the large number of patients with UTI and our deficient knowledge of how to handle them in many respects—especially how to prevent renal scarring.

We do know, however, that a serum antibody response is regularly detectable against the O antigen of the infecting *Esch. coli* (Winberg *et al.*, 1963). This response appears in patients with acute pyelonephritis but not cystitis when the indirect haemagglutination assay is used. With other techniques such as direct agglutination, the Farr

technique, or the enzyme-linked immunosorbent assay, slightly differing results may be noted due to the influence of immunoglobulin class and avidity of the antibodies on the capacity of the various methods to measure them (Ahlstedt, Holmgren, and Hanson, 1972; Ahlstedt *et al.*, 1975b). With the ammonium sulphate precipitation technique of Farr, increased antibody levels can actually be picked up in some cases of acute cystitis, possibly those with a pronounced tissue engagement (Ahlstedt, Jodal, and Hanson, 1975c).

In contrast to the commonly encountered O antibody response, antibodies to the K antigens of the infecting bacteria are less consistently present. The K1 antigen, which is the most common capsular antigen among *Esch. coli* causing acute pyelonephritis in particular (39%) and UTI in general (28%; Kaijser, Hanson, and Robbins, 1976), seems to induce nonresponsiveness in most patients with acute pyelonephritis (Kaijser, Jodal, and Hanson, 1973). It is still unknown whether or not this has consequences for the clinical appearance and course of the disease. In addition to the serum antibody response there also seems to be a local antibody formation in the urinary tract against *Esch. coli* O and K antigens demonstrable in the urine especially as SIgA but also as IgG antibodies (Jodal *et al.*, 1974; Hanson *et al.*, 1975a).

From experimental infections in animals it seems as if IgG as well as IgM antibodies against the O and especially the K antigen of the *Esch. coli* are protective (Kaijser, Holmgren, and Hanson, 1972; Kaijser and Olling, 1973). This is true for haematogenous pyelonephritis and also, but at a lower level, for ascending pyelonephritis after parenteral and even intravesicular vaccination with killed bacteria (Kaijser and Larsson, 1976; Hanson *et al.*, 1976b). Finally we have recently noticed that *Esch. coli* strains isolated from the urine of patients with pyelonephritis adhere to epithelial cells from the normal human urinary tract much more efficiently than bacteria isolated from the urine of patients with asymptomatic bacteriuria (ABU) (Table II). This adherence seems to be prevented *in vitro* by urine antibodies against the bacteria (Hanson *et al.*, 1976b; Svanborg Edén *et al.*, 1976).

There is only indirect evidence that the serum and urine antibodies against the infecting *Esch. coli* can affect the appearance and course of the disease in patients with UTI. Thus recurrences are in 80% caused by bacteria of a serotype different from that of the bacteria causing the preceding infection (Bergström *et al.*, 1967). Furthermore, continuous changes of surface characteristics seem to occur in *Esch. coli* isolated from the urine of longitudinally

TABLE II

Capacity to attach to normal uroepithelial cells of *Esch. coli* isolated from urine of patients with various forms of urinary tract infection

	Origin of <i>Esch. coli</i>		
	Acute pyelonephritis	Acute cystitis	Asymptomatic bacteriuria
No. of strains tested	22	8	22
No. of bacteria/epithelial cells			
Mean	44	26	2
SD	41	37	4

For details see Svanborg-Edén *et al.*, 1976

followed untreated patients with ABU (Lindberg *et al.*, 1975c; Hanson *et al.*, 1976b). This possible 'antigenic drift' of the bacteria may well be induced by the local immune response in the form of SIgA antibodies. These antibodies may exert their function by binding the bacteria, thereby preventing them from attaching to the epithelial cells. This may be sufficient to prevent tissue invasion and symptom-causing infection. Furthermore, the changes of the *Esch. coli* to the forms typical for ABU seem to result in less virulent bacterial forms with 45% being spontaneously agglutinating—less than 2% of such *Esch. coli* are found in patients with acute pyelonephritis (Lindberg *et al.*, 1975d; Hanson *et al.*, 1976b). This may be another reason why there are so few or no symptoms in ABU. Thus, the adjustment of the micro-organisms in the urine to the host defence may have the clinically significant effect of a less harmful infection for the host. If this assumption holds true it has consequences for our evaluation of the needs for investigation, treatment, and follow-up of ABU patients.

Although 93% of patients with ABU can be successfully treated by a short course of nitrofurantoin, in 52% it will recur within 3 years and in 22% it will recur repeatedly (Lindberg, Jodal, and Hanson, 1976). In contrast, 30% of untreated ABU patients will rid themselves of the bacteria spontaneously, and during a 3-year follow-up there were no sign of effects on renal function or appearance on intravenous urography or micturition cystourethrography in patients still bacteriuric (Claesson and Lindberg, 1976). There may be reason not to treat all ABU patients if a consequence of the 'antigenic drift' we advocate for the *Esch. coli* of ABU patients is a less harmful, often spontaneously vanishing infection which gives little or no symptoms (Lindberg *et al.*, 1975c; McLachlan *et al.*, 1975). We need, however,

studies to make sure that we do not thereby miss patients who may develop renal parenchymal reduction, perhaps due to the presence of severe reflux, residual urine (Lindberg *et al.*, 1975a), or other mechanical factors. We need simple techniques to detect patients at risk for renal scarring (Winberg *et al.*, 1974) and eventual lowered renal function (Peterson *et al.*, 1976). One method which holds promise for detecting patients with renal parenchymal involvement is the demonstration of increased levels of serum IgG auto-antibodies to the Tamm-Horsfall protein normally present in renal tubules and urine (Hanson, Fasth, and Jodal, 1976c). Another very simple but less specific pointer to renal involvement is the presence of increased levels of C-reactive protein in the blood (Jodal, Lindberg, and Lincoln, 1975; Jodal and Hanson, 1976).

Although information is accumulating which may become useful in the handling of patients with UTI, it is obvious that our knowledge is still inadequate.

Summary

The *Esch. coli* harboured in the gut constitute a reservoir of potential pathogens in the infant and child. The conditions required for these intestinal inhabitants to cause infection are not well understood. The presence of virulence factors such as capsular antigens, especially K1, may be of significance for the ability of *Esch. coli* to cause neonatal meningitis. The capacity of certain *Esch. coli* to attach to epithelial cells of mucous membranes may be important for their infective powers in the urinary as well as the intestinal tract. Furthermore, the ability of certain *Esch. coli* to produce enterotoxins similar to that of *V. cholerae* is of importance for their capacity to provoke diarrhoea.

The importance of the immune defence mechanisms for prevention of these *Esch. coli* infections is suggested, especially in the form of local immunity provided by secretory IgA antibodies. Such antibodies directed against *Esch. coli* O and K antigens as well as enterotoxins are present in large amounts in human milk and may be of considerable importance for protection against *Esch. coli* in the breast-fed baby. Breast feeding may be of special significance until the baby has built up its own local immune defence preventing the micro-organisms from attaching to and invading the intestinal mucous membranes. SIgA antibodies in urine may have a similar protective effect against urinary tract infections. The variable pictures of *Esch. coli* infections in childhood are striking, ranging from severe sepsis/meningitis or diarrhoea to 'asymptomatic' bacteriuria. This variability is

obviously closely connected with the presence of various virulence factors and the function of different components of the immune defence.

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