model of spontaneous gastric perforation should allow these other factors to be evaluated.

SUMMARY

Spontaneous gastric perforations usually occur in well infants during the first 5 days of life. This study shows that a well infant ingests an average of 360 ml air in 6 hr. This amount is sufficient to cause gastric rupture if entrapped in the stomach and not allowed to escape through the esophagus or small bowel. An experimental model utilizing eight rodents and six puppies showed that fluid could prevent the movement of air distally into the small bowel. The change of angle at the gastroesophageal junction when the gastric antrum was distended with air prevented proximal reflux. Rupture was produced in the eight rodents and in the three puppies less than 10 days of age but not in the puppies older than 10 days.

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A Review: Relation between Invasiveness and the K1 Capsular Polysaccharide of *Escherichia coli*

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Under certain conditions, *Escherichia coli* may invade the blood and localize in various tissues. The host factors and bacterial properties related to the penetration of *E. coli* through its usual supramucosal localization in the intestinal tract or from other sites are incompletely understood. Recently, a direct association between an invasive bacterial disease, neonatal meningitis, and a single *E. coli* structure, the K1 antigen, has been reported (44). Our studies of the relation to disease and characteristics of the K1 antigen causing neonatal meningitis in this comparatively homogeneous patient population provide some insight into the mechanisms of *E. coli* invasiveness.

The term K antigens of *E. coli*, first introduced by Kauffmann and Valhne (32), was intended to designate an envelope or surface structure. Most of the 100 categorized K antigens (International Centre for Reference and Research on Escherichia, World Health Organization) are capsular polysaccharides, similar in their overall chemical, physical, and morphologic structure to the capsular polysaccharides of highly invasive bacteria such as meningococcus, pneumococcus, and *Haemophilus influenzae* type b (4, 37, 38). Originally based upon serologic reactivity, more exact biochemical and genetic information has shown that the K88 and K89 antigens are fimbriae or pili (episomal-directed proteins) that serve as attachment factors to mammalian cells (54) and that K80 antigen is a component of the somatic or O antigen (25).

E. coli K capsular polysaccharides are linear co- or homopolymers composed of one or several monosaccharides joined by glycosidic and/or phosphodiester bonds. A neutral pH, they have a net negative charge because of these phosphodiester bonds or uronic acid components. The relation between capsular polysaccharides and *E. coli* invasiveness was first shown by Smith (52) and later by Kauffman (31). Surveillance data has shown that *E. coli*, isolated from extraintestinal sites, have acidic capsular polysaccharides (39). These invasive strains are found in normal intestinal tract along with a great variety of non-K antigen bearing *E. coli*. Further studies demonstrated that K antigens, isolated from *E. coli* that had infected renal parenchyma, had a higher molecular weight

and were in higher concentration per bacterial cell than K antigens from strains of the same serotype confined to the bladder or intestinal tract (14, 26, 39). The mechanism by which the K capsular polysaccharides conferred renal parenchymal invasiveness to *E. coli* strains was postulated to be their anticomplementary effect (14, 15). Further, some *E. coli* K antigens are immunochemically related to the capsular polysaccharides of highly invasive bacteria, including *Neisseria meningitidis* group B and group C, *Diplococcus pneumoniae* types 1, 2, 4, 7, 10, and 23 and *H. influenzae* type b (5, 17, 21, 45, 50, 61). This evidence suggests that the K capsular polysaccharides are important determinants of invasive *E. coli* disease.

NEONATAL *E. COLI* MENINGITIS

Human and animal neonates are particularly susceptible to *E. coli* septicemic diseases, including meningitis (6, 19). Despite advances in antimicrobial chemotherapy, the combine mortality and morbidity of neonatal meningitis and septicemia remains high: there are few normal survivors (28, 19, 34, 35, 41, 57). There is little information regarding the antigenic, metabolic characteristics and origin of cerebral spinal fluid (CSF) *E. coli* related to the pathogenesis of neonatal meningitis. The *Escherichia* genus is antigenically quite complex, comprising some 155 somatic (O), 100 capsular (K), and 50 flagella (H) antigens. Curiously, there have been no reported descriptions of serotypes of CSF isolates from newborns. Based upon the high specific activity of IgM antibodies as compared with IgG antibodies in several biologic assays, including complement-dependent bactericidal reaction and opsonization and protection tests, several investigators proposed that the deficiency of this immunoglobulin in the newborn was related to increased susceptibility of infants to gram-negative infections (12). In addition, other developmental deficiencies, such as diminished serum complement proteins, low levels of opsonins, and lesser phagocytic and bactericidal activity of lymphoid cells, have been implicated (36, 51, 55, 56). However, an association between the *E. coli* K1 capsular polysaccharide and neonatal meningitis should direct attention to the organism as well as the host to account for newborn susceptibility toward *E. coli* septicemia and meningitis (44).

UNUSUAL CHARACTERISTICS OF *E. COLI* K1 ANTIGEN

Grados and Ewing (17) reported that an *E. coli* isolated from the spinal fluid of a newborn agglutinated with meningococcus group B antiserum. More detailed studies by Kasper *et al.* (30) showed that the shared antigen between meningococcus group B and the *E. coli* strain O7:K1(L):NM, reported by Grados and Ewing, was the capsular polysaccharide antigens of these two bacterial species. The chemical composition of the *E. coli* K1 polysaccharide, first described by Barry and Goebel (2, 3), is a polymer of *N*-acetylneuraminic acid. Further studies have shown that this *E. coli* polysaccharide was structurally similiar or identical with the meningococcal group B polysaccharide (33). In addition to their structural similiarity, both the purified *E. coli* K1 and meningococcus group B polysaccharides will induce the formation of serum antibodies in most young adults (33). When injected as whole formaldehyde-treated encapsulated organisms into laboratory animals, both meningococcus group B and *E. coli* fail to induce the usual amount of serum anticapsular antibodies elicited by other encapsulated bacteria. In contrast to meningococcus groups A and C, asymptomatic nasopharyngeal carriage of meningococcus group B did not elicit anti-group B polysaccharide antibodies (61). Furthermore, only a small fraction of adults recovering from meningococcal group B septicemic diseases, including meningitis, responded with the expected level of serum anticapsular antibodies as compared with patients convalescent from meningococcal group A or group C meningitis (7). Although the clinical setting is not strictly comparable, most children synthesize anti-*O* but not anti-K1 antibodies after urninary tract infections with these organisms (29).

PREVALENCE OF K1 ANTIGEN AMONG CSF AND BLOOD ISOLATES FROM HUMAN NEWBORNS WITH *E. COLI* DISEASE

The prevalence of the K1 antigen among *E. coli* isolates from neonates with invasive diseases including meningitis and from diseased and healthy individuals of all ages was compared. Among 163 neonates with *E. coli* meningitis, 126 (77%) had K1-containing organisms. These organisms were CSF isolates supplied to us by participating members of The Cooperative Neonatal Meningitis Study Group, the Department of Pediatrics at the University of Goteborg, Sweden, and by many other cooperating institutions and physicians directly concerned with the care of these patients (34, 44). Similar results were obtained by studying the organisms submitted to the Enterobacteriology Laboratory, Center for Disease Control, from the years 1967 through 1973 (Table 1).

A direct relation between the K1 antigen and meningitis was shown by the observation that only 40% of *E. coli* blood isolates in infants with septicemia without meningitis were K1 strains. This might be explained by the fact that K1-containing strains are more invasive, can persist in the circulation longer and in higher concentrations, and can localize ultimately in the meninges. A similiar tendency for K1 to be found more often in *E. coli* infections in children as adults was observed among urinary tract isolates. Approximately 20% of urinary *E. coli* from children were K1 in contrast to approximately 10% K1 strains found among urinary tract isolates in adults including pregnant women (29).

ANTIGENIC AND METABOLIC CHARACTERISTICS OF CSF *E. COLI* K1 STRAINS

The most frequently encountered somatic antigens of the CSF K1 isolates were O18ac, O7, O1, and O16. These *O* antigens comprise about two thirds of the CSF K1 strains (46). Other CSF K1 O serotypes were *O*132, *O*123, *O*83, *O*134, *O*156. A significant proportion of the CSF K1 isolates spontaneously agglutinated, presumably because of deficient *O* polysaccharide side chains of their somatic antigens. H7 and H6 were the most common flagellar antigens although at least half the CSF strains could not be typed with available antisera. Late lactose fermentation, an unusual biochemical characteristic of *E. coli*, was observed among 15% of K1 strains isolated from healthy and diseased individuals. One of the CSF *E. coli* strains reacted with meningococcus C. This organism, strain N67 *O*13:K?:NM also reacts with equine meningococcus group B antiserum and will be the subject of a future report. No CSF organisms reacted with pneumococcus type 1 and 3 or *H. influenzae* type b antiserum. One strain isolated from the blood of the neonate without meningitis cross-reacted with pneumococcus type 3, indicating it had the K7 capsular polysaccharide

Table 1. *Prevalence of K1 antigen among neonatal cerebral spinal fluid (CSF) and blood E. coli isolates sent to enterobacteriology laboratory, Center for Disease Control, 1968–1973*[1]

Source of organism	K1 positive	K1 negative
CSF	12[2]	3
Blood (presence or absence of meningitis not stated)	15[2]	10
Miscellaneous CSF isolates[3]	0	2
Myelomeningocoele patients (CSF)	1	2

[1] The organisms were identified as K1 by immunodiffusion of *E. coli* saline extracts with equine meningococcal group B antiserum. The organisms were kindly supplied by Dr. George Hermann, Center for Disease Control.

[2] One K1 CSF strain and three K1 blood isolates, all of the same O and H serotype, were sent from different patients at the same hospital at the same time.

[3] From older children whose underlying disease was not stated.

(46). There was no association between the K1 capsular polysaccharide and other *E. coli* structures such as the *O* and H antigens.

Recently, it has been shown that outer membrane proteins of encapsulated meningococci are related to their disease potential (11, 16, 22, 49). Furthermore, serum bactericidal activity, detectable after nasopharyngeal carriage or disease with meningococcal group B organisms, is due to antibodies directed toward outer membrane proteins. These proteins exhibit polymorphism which has formed the basis for a typing scheme (11, 16). Although there are at least 12 different menigococcal group B outer membrane proteins, types 2 and 9 account for about 85% of the meningitis isolates. In contrast, at least 9 other serotypes have been isolated from asymptomatic carriers. The demonstration of these heterogeneous outer membrane proteins from *E. coli* by Schnaitman (49) suggests that these antigens should be studied from K1 strains isolated from various sources.

The capsular polysaccharide content of K1 *E. coli* from the CSF, blood, and stools of healthy and sick neonates and the blood and stools of adults were assayed for their *N*-acetylneuraminic acid content by the thiobarituric reaction and for their immunologically reactive content by the Mancini technique using meningococcus group B antiserum. These experiments showed that the K1 capsular content was similar among strains isolated from the CSF and blood of sick neonates or the stools of healthy infants, children, and adults, suggesting that the total amount of encapsulation was not related to virulence. Thus, it is not possible to recognize a "virulent" *E. coli* K1 strain by the determination of its capsular content (47).

VIRULENCE OF *E. COLI* K1 STRAINS

Despite the use of antimicrobials, the mortality of neonatal *E. coli* meningitis is approximately 35% and morbidity among survivors is approximately 50% (18, 19, 34, 35, 41, 57). K1 strains are highly virulent for mice (59). Accordingly LD_{50} values for 43 K1 and 9 non-K1 CSF isolates in a mucin-enhanced, lethal mouse infection assay were compared (34, 44). The mean LD_{50} for the K1 organisms was 168 (range 2–800) and the mean for the non-K1 strains was 5.8×10^4 (range 790–3.9×10^5). No differences in antibiotic sensitivities were observed among the K1 or K1-negative CSF strains.

RELATIONSHIP BETWEEN CONCENTRATION AND PERSISTENCE OF SERUM AND CSF K1 ANTIGEN AND MORTALITY AND MORBIDITY OF NEONATAL MENINGITIS

To study further the relationship between the K1 antigen concentration and the pathogenesis of neonatal *E. coli* meningitis, the presence and the of the K1 antigen in serum and CSF of patients was studied using countercurrent immunoelectrophoresis (CIE) (34). CSF specimens from 41 of the 48 infants with *E. coli* K meningitis were available. The K1 antigen was detected in the CSF from 29 of 41 infants (71%). With the exception of 4 infants, CSF cultures were positive 1–4 days longer than CSF K1 antigen was detectable. Higher K1 antigen levels and longer duration of detection were present among those who succumbed to the disease or had an abnormal outcome. Among the group without detectable neurologic abnormalities only half had CSF K1 antigen. Their level was lower and the duration of antigenemia was shorter. A similiar direct relation was obtained for the clinical outcome and serum K1 antigen concentration. Of those infants with a normal outcome, 3 of 14 had detectable K1 antigen as contrasted to 8 of 11 with detectable serum K1 antigen who succumbed.

The mortality of those with K1 meningitis was significantly higher than those with non-K1 disease; their cultures remained positive longer and they had a higher frequency of relapse after "successful" antimicrobial chemotherapy. As has been shown for *H. influenzae* type b (1, 24, 58) *D. pneumoniae* (10, 23), *N. meningitidis* (8, 9), and *Klebsiella pneumoniae* (42), a direct relation was also demonstrable between the CSF and or serum capsule concentration and the mortality and morbidity in neonatal *E. coli* meningitis. The CIE assay may prove to be a valuable diagnostic test and provide information for prognosis and therapy for neonatal *E. coli* meningitis.

Invasiveness of *E. coli* K1 strains was investigated in a HeLa cell *in vitro* model and in the conjunctiva of rabbits (48). K1 organisms were not invasive by these two assay systems as contrasted to *Shigella* strains which penetrated the HeLa cells and caused purulent conjunctivitis and corneal scarring in rabbits.

IN VIVO EFFECT OF K1 POLYSACCHARIDE INJECTION IN MICE

The possibility that the circulating K1 antigen exerted a nonspecific depression of resistance mechanisms was examined in a mucin-enhanced, *E. coli* K1 lethal infection in mice. The K1 polysaccharide was purified from two *E. coli* strains, *O7* and *O64*. The serum half-life of the K1 antigen in 15–18-g, all-purpose NIH mice, was determined by measuring the concentration by radial immunodiffusion at various intervals after intravenous injection of 0.5 and 1.5 mg of the purified antigen. The half-life of the intravenously injected K1 polysaccharide was approximately 8.0 hr. The LD_{50} of two K1 strains was not affected by injecting 0.5 mg K1 antigen immediately or at various intervals up to 2 weeks before challenge with the mucin-suspended *E. coli* (Table 2).

The purified K1 polysaccharide did not induce protection. It was concluded that *E. coli* virulence in mice was not related to serum K1 antigen; this indicated that the capsular polysaccharide, *per se*, is not a general inhibitor of resistance mechanisms.

MECHANISMS OF IMMUNITY

Anticapsular antibodies confer immunity to diseases caused by *H. influenzae* type b, meningococci, and pneumococci. To study the role of anti-K1 antibodies, goat meningococcal group B antiserum was applied to a meningococcal group B polysaccharide-sepharose 4B derivative and the adsorbed K1 antibodies purified by elution with 3 M KCNS. Use of the purified anticapsular antibodies avoids the problem of interpreting results with antisera prepared by injection of whole *E. coli* with their many noncapsular, cross-reacting antigen-antibody systems. A highly protective effect of this purified meningococcus group B antibody with K1 activity was observed with gastric mucin or saline suspensions *E. coli* in a mouse lethal infection model (44). The specificity of this anti-K1 antibody was verified by its lack of effect upon the LD_{50} of non-K1 strains. Wolberg and deWitt (59) and Kaijser (27) have shown that K1 antibodies, induced by the injection of whole *E. coli* organisms, will induce protection. Thus, immunity to neonatal *E. coli* meningitis may be mediated by anti-capsular antibodies. The source of these proposed protective K1 antibodies in newborns may be maternal serum or colostrum.

Table 2. *Mouse LD_{50} of Escherichia coli O62:K1:H6 challenge after intravenous injection of purified K1 polysaccharide*[1]

Time after K1 injection	LD_{50}	
	K1 injected	Controls
0.5 hr	3.6×10^3	2.35×10^3
24 hr	1.45×10^2	1.08×10^2
18 days	2.82×10^3	0.89×10^3

[1] Five groups of five NIH-all purpose 15–20-g mice each were injected intravenously with 0.5 mg purified *E. coli* K1 polysaccharide. They were then challenged with *E. coli* O62:H1:H6 suspended in mucin and the LD_{50} calculated according to the method of Reed and Muench (43).

PATHOGENESIS OF DISEASE

There are similarities between neonatal *E. coli* meningitis and meningitis in children and adults caused by the usual pathogenic encapsulated bacteria. First, these pathogens are encapsulated with an acidic polysaccharide. Second, a protective effect of "natural" and/or immunization-induced anticapsular antibodies can be demonstrated. Third, the mortality and morbidity of meningitis is related to the concentration and persistence of capsular polysaccharides in the CSF and/or the blood. With the exception of epidemics, the nasopharyngeal carriage or virulent types of meningococci, *H. influenzae* type b and pneumococci is quite low. Accordingly, studies were undertaken to determine the frequency with which *E. coli* K1 strains could be found in the normal gastrointestinal flora and other sites of newborns, mothers, and hospital personnel (47). *E. coli* strains were collected from urine cultures, rectal swabs from healthy newborns, CSF cultures of neonates with meningitis and, where possible, stool cultures from these patients, their mothers, and hospital personnel, rectal swabs from pregnant women at delivery, and nonpregnant females from family planning clinics and individuals of all ages from outpatient departments at various hospitals. The detection of the K1 strains was done by the antiserum agar technique using equine meningococcus group B antiserum prepared by intravenous injection of formaldehyde-fixed meningococcus group B (strain B11). This antiserum contained approximately 0.5 mg anti-K1 capsular polysaccharide antibody/ml. Swabs were streaked onto antiserum agar plates and incubated overnight at 37°. After initial examination for halos of precipitation surrounding the individual colonies, the plates were incubated for an additional 24 hr at 4° and reinspected using a high intensitiy spotlight against a dark background. Analysis of 664 rectal swab cultures, positive for *E. coli* K1 strains, revealed pure or almost pure growth in 35%, moderately heavy growth (greater than 10 of *E. coli* K1 colonies) in 31%, and less than 10 halo-producing colonies in the remainder of the cultures. The antiserum agar technique was compared with the conventional procedure of picking isolated colonies and K typing by slide agglutination and/or immunoelectrophoresis. It was found that in the cultures with a light growth a higher yield of K1-positive organisms was achieved with the antiserum agar plates. In those swabs where the K1 organism was predominant, there was no significant difference between the two techniques. Therefore, the antiserum agar technique seemed to be more sensitive in spotting K1 organisms than analysis of individual colonies from standard media. Further, *E. coli* are usually identified by 24-hr lactose fermentation on conventional media. The reliance on this fermentation property may result in missing many K1 strains that are late lactose fermentors (47). In healthy newborns, *E. coli* K1 strains were found in approximately one-fifth of newborns (range 7 38%). Prevalence rates within the same nursery at different times were variable. For instance, K1 strains were detected in only 1 of 100 *E. coli* from neonates in Mexico City in December 1973 and May 1974. However, 7 of 20 (35%) had K-positive strains when this nursery was restudied in October 1974. That same variation was observed in the premature nursery at Parkland Memorial Hospital. Despite no obvious changes in the routine handling of these babies or in nursing personnel, during the first 19 weeks 10 32% of the infants had detectable *E. coli* K1 in contrast to the next 8 weeks, where the K1 prevalence rate fell to 0–11% (mean 6.6%). In the last 4 weeks of the study, K1 strains were found in 11 16% (mean 13.5%) of these rectal swabs. The reason for this variability in the *E. coli* K1 prevalence rates in stool cultures in an individual nursery was unknown. In a study of individuals varying in age from premature infants to adults, including pregnant women, it was found that approximately 20 40% of individuals possessed *E. coli* K1 strains. Acquisition of *E. coli* K1 in healthy term infants was directly related to the presence of the same organism in the maternal stool. Among 97 mothers studied for K1 organisms at delivery, 44% had *E. coli* strains. Two-thirds of neonates born to these K1-positive mothers had K1 strains, whereas only 11% of infants born to K1-negative mothers had K1-positive stool cultures. Among full term babies who eventually acquired *E. coli* K1, 77% had positive rectal cultures by the second day and 91% by the third day. Studies comparing the time of acquisition by infants born to K1-positive mothers suggested that K1 strains were acquired by these babies more rapidly than those infants who had K1 in their stools when discharged from the hospital but whose mothers were K1 negative. Term infants acquired their K1 strains more rapidly than premature infants, who were kept in strict isolation. This latter observation suggests that mothers are the chief source of K1 organisms for most newborns. However, K1 strains can be acquired from nonmaternal sources, such as hospital personnel.

E. coli flagella (H) and somatic (*O*) serotypes of the K1 strains from normal stools were compared with those strains isolated from the CSF. To date, our conclusion is that there is no difference in the prevalence rate of H and O serotypes of K1 strains isolated from the CSF when compared with K1 strains taken from stools of healthy individuals.

Rectal swab cultures were obtained from 17 mothers whose infants had *E. coli* K1 meningitis; 11 mothers had the same O and H serotype of *E. coli* as that causing the meningitis. In an additional mother-infant pair, *E. coli* *O*83:K1:H2 was cultured from multiple sites of the infant and from the maternal urine but a different serotype was isolated from the maternal rectal swab. These studies indicate that in *E. coli* K1 meningitis, the offending organism can be isolated from the mother, strongly suggesting that this is the direct source.

Thus, in contrast to the low carriage rate of the virulent capsular types of pneumococci, meningococci, and *H. influenzae* type b, *E. coli* K1 organisms are detected in approximately 20–30% of individuals of all ages. Furthermore, K1 strains are the predominant organisms in those infants have K1-positive rectal swabs. Accordingly, differences in the rate and degree of exposure do not adequately explain the age relationship of *E. coli* K1 meningitis in the neonatal period. Similar K1 prevalence rates in newborns have been reported by Ørskov and Sorensen (40). Assay of serum K1 antibodies, using the ELISA technique, has shown that this antibody is infrequently detected in the serum of normal adults. Further, in the goat and horse sera prepared by repeated injection of formaldehyde-treated meningococcus group B organisms, virtually all the detectable antibody was of the IgM type (13). Kaijser and Hanson (28) have found that K1 antibodies are prevalent in colostral IgA. These preliminary experimental data suggest that colostral anticapsular antibodies may exert a different protective role in the pathogenesis of *E. coli* disease in neonatal meningitis than the role mediated by serum antibodies in children and adults.

The K1 is the most frequent capsular antigen serotype found in urinary tract infections in infants and children (26). Furthermore, approximately 10–15% of *E. coli* blood cultures in adults are K1 organisms. Thus, the mechanisms of invasiveness and immunity that are critical in determining the pathogenesis of neonatal *E. coli* meningitis should provide important information in understanding other common and serious *E. coli* diseases.

SUMMARY

The conclusions from our studies to date may be summarized as follows. (*1*) Invasive *E. coli* strains causing neonatal meningitis are encapsulated. At least 80% of those strains inducing mengitis are K1 and approximately 40% of those strains isolated from infants with septicemia but without meningitis are also K1. Invasiveness is best related to the K1 antigen and not to *E. coli* O and H antigens. (*2*) The capsular content of CSF strains is not related to their invasiveness. In contrast to observations reporting higher K capsular polysaccharide content and molecular weight of *E. coli* invading the renal parenychma as compared with those *E. coli*

confined to the bladder or in the stool, there were no differences among CSF K1 strains. Speculation as to the mechanism of the invasive properties conferred by acidic capsular polysaccharides may be derived from the literature. Unencapsulated or "rough bacteria" are susceptible to the bactericidal action of agammaglobulinemic sera (15, 53). When injected into precolostral (agammaglobulinemic but complement containing), cesarian-delivered, and antigen-deprived piglets, unencapsulated bacteria are rapidly cleared from the circulation. In contrast, smooth bacteria injected into these same animals circulate without detectable splenic or hepatic clearance, multiply, and result in the death of these animals. The mechanism of the resistance of encapsulated bacteria has been postulated to be due to the inaccessibility of the deep somatic antigen structures capable of activating the alternate complement pathway system. Thus, opsoninization and other host complement-dependent activities may of necessity be antibody mediated for encapsulated bacteria. This complement resistance of encapsulated organisms may be quanititative and studies should be done to determine differences among various K1 *E. coli* strains. (*3*) K1 strains are widely prevalent among infants, children, and adults and are quickly transmitted to infants. In most cases the source of the infecting strain in diseased infants is the mother. However, transmission from attendants, demonstrable in our studies, is also a possible mechanism. (*4*) A protective role of serum anticapsular antibodies in animal models has been demonstrated. Our initial observations indicating low serum K1 antibodies in the general population and the finding that K1 antibodies are predominantly IgM in two animal species studied so far suggest that colostral K1 antibodies may be important in conferring immunity to this disease.

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Cystic fibrosis
genetic disease
isoelectric focusing

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protease inhibition

Studies on Cystic Fibrosis Using Isoelectric Focusing. II. Demonstration of Deficient Proteolytic Cleavage of α_2-Macroglobulin in Cystic Fibrosis Plasma

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Extract

A protein with an isoelectric point (pI) of 5.48 was found to be deficient in plasma from most cystic fibrosis (CF) homozygotes and obligate heterozygote carriers for CF as compared with normal control plasma. Purification of the protein with a pI of 5.48 from normal plasma was performed using ammonium sulfate precipitation, DEAE-cellulose and CM-cellulose chromatography, Sephadex G-200 gel filtration, starch block electrophoresis, and Sepharose 4B gel filtration. The purified protein migrated as a single band on polyacrylamide gel electrophoresis, and displayed a single arc on immunoelectrophoresis against polyvalent antiserum to whole human serum. Results from various techniques used in its characterization indicate that this protein is a fragment of α_2-macroglobulin (α_2M) which is derived from α_2M by proteolytic cleavage of intact α_2M subunits. Quantitation of α_2M levels in plasma indicated no significant differences between levels of α_2M in CF homozygote, obligate heterozygote carrier, or normal control plasma samples. Quantitation of arginine esterase activity in plasma treated with chloroform and ellagic acid indicated that both the total arginine esterase activity and that fraction of arginine esterase activity inhibited by soybean trypsin inhibitor (SBTI) were decreased in most CF homozygote and obligate heterozygote plasma samples relative to normal control values. The results of this study indicate that plasma samples from CF homozygotes and obligate heterozygote carriers for CF show deficient proteolytic cleavage of α_2M as compared with normal control plasma, and suggest that a structural abnormality in α_2M or a deficiency in plasma proteolytic activity may be responsible for this deficiency in proteolysis.

Speculation

An abnormality in the binding affinity of α_2M for plasma proteases may account for the presence of "factors" in CF homozygotes and obligate heterozygote carriers.

Cystic fibrosis is a generalized metabolic disorder, resulting from an unknown genetic defect (33). It is generally thought to be transmitted as a single autosomal recessive trait (10, 33), although several authors have found evidence for genetic heterogeneity within the clinical entity known as CF (38, 53) and have suggested that the disease may actually be a group of closely related genetic abnormalities with similar pathologic consequences (53). Several reports have indicated that sera from patients with CF (CF homozygotes) and from obligate heterozygote carriers contain factors that may be characteristic of the disease and thus possibly related to the primary genetic defect in CF (7, 11, 50, 52, 53). Other factors are found in saliva and sweat from CF homozygotes (31) and have been produced from cultured cells derived from CF homozygotes and heterozygotes (4, 6, 13). Partial characterization of the various CF factors suggests that they are closely related