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Studies on Immunity Against Escherichia coli K13 with Monoclonal Anti-K13 and Anti-anti-K13

Summary: The structural basis for the cross-reactivity between the *Escherichia coli* K13, K20 and K23 capsular polysaccharides is the →)-β-ribofuranosyl-(1→7)-β-2-keto-3-deoxyoctonate polymer. Monoclonal antibodies against *E. coli* K13 which require O-acetyl-2-keto-3-deoxyoctonate for binding were further investigated. Such antibodies, of both the IgG and the IgM isotype, opsonized *E. coli* K13 *in vitro* and protected against intraperitoneal infection in mice as well as ascending pyelonephritis in rats. A monoclonal IgG1 anti-idiotype, specific for the K13 polysaccharide combining site of a protective IgM idiotype, primed for protection against intraperitoneal infection with live *E. coli* K13 following K13 injections at four as well as 12 weeks of age. the K13 polysaccharide alone did not immunize and protect. The monoclonal anti-K13 idiotype only primed for protection at four weeks of age. These findings suggest a strong effect of a single idiotype on the outcome of a bacterial infection.

Zusammenfassung: *Untersuchungen zur Immunität gegen Escherichia coli K13 mit monoklonalem Anti-K13 und Anti-anti-K13.* Das →)-β-ribofuranosyl-(1→7)-β-2-keto-3-deoxyoctonat-Polymer stellt die strukturelle Basis für die Kreuzreaktivität zwischen den *Escherichia coli* K13-, K20- und K23-Kapselpolysacchariden dar. Es wurden Untersuchungen an monoklonalen Antikörpern gegen *E. coli* K13 durchgeführt, die für die Bindung O-acetyl-2-keto-3-deoxyoctonat benötigen. Solche Antikörper, sowohl vom IgG- wie vom IgM-Isotyp, opsonierten *E. coli* K 13 *in vitro* und besaßen protektive Wirkung gegen die intraperitoneale Infektion bei Mäusen und die aszendierende Pyelonephritis bei Ratten. Ein monoklonaler IgG1 anti-Idiotyp mit Spezifität gegen die K13-Polysaccharid-Bindungsstelle eines protektiven IgM-Idiotypen prägte eine Protektion gegen die intraperitoneale Infektion mit lebenden *E. coli* K13 nach K13-Injektion im Alter von vier und von 12 Lebenswochen. Das K13-Polysaccharid allein führte nicht zur Immunisierung und Protektion. Der monoklonale Anti-K13-Idiotyp prägte nur im Alter von vier Lebenswochen eine Protektion. Aus diesen Befunden läßt sich ableiten, daß ein einzelner Idiotyp den Ausgang einer bakteriellen Infektion wesentlich beeinflußt.

Serological Studies on Escherichia coli K13 with Monoclonal Antibodies

Escherichia coli with the K13 capsular polysaccharide (K13 Ps) are among the most common pathogens in human urinary tract infections (1, 2). The composition of the K13 Ps was described by *Vann* and *Jann* (3) and *Vann* et al. (4).

Monoclonal antibodies against the K13 Ps were utilized to examine the cross-reactions between the *E. coli* K13, K20 and K23 Ps (5) and to study the opsonizing and protective capacity of defined capsular antibodies.

The monoclonal antibodies were made by fusion of spleen cells from Balb/c mice immunized with the K13 Ps covalently linked to bovine serum albumin (K13-BSA) with the SP2/0 mouse plasmacytoma line (6). Antibody-producing clones were tested by the enzyme-linked immunosorbent assay (ELISA) coating the polyvinyl microtiter plates (Dynatech Laboratories, Va, U.S.A.) with K13 Ps (7). The monoclonal antibodies were tested for isotype using ^{3}H-labeled subclass-specific antibodies in a solid phase radioimmunoassay; they were studied by isoelectric focusing (6, 8).

Antibodies were obtained that could distinguish the K13, K20 and K23 Ps by immunodiffusion analysis (4). The polysaccharide cross-reactivity was further studied by counter immunoelectrophoresis (4, 9). Structural analyses, including ^{13}C-NMR, ^{1}H-NMR and gas-liquid chromatography-mass spectrometry, confirmed the results of the serological studies and suggested the polysaccharide compositions shown in Table 1.

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Table 1: The composition of the *Escherichia coli* K13, K20 and K23 capsular polysaccharides.
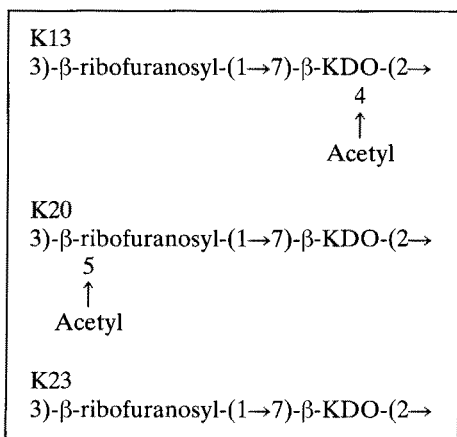
K13
3)-β-ribofuranosyl-(1→7)-β-KDO-(2→
$$\begin{array}{c}4\\\uparrow\\\text{Acetyl}\end{array}$$

K20
3)-β-ribofuranosyl-(1→7)-β-KDO-(2→
$$\begin{array}{c}5\\\uparrow\\\text{Acetyl}\end{array}$$

K23
3)-β-ribofuranosyl-(1→7)-β-KDO-(2→

Table 2: Interaction between *Escherichia coli* K13 and human granulocytes in the presence of monoclonal anti-K13 and anti-anti-K13 (anti-idiotype).

Escherichia coli K13 and antibodies	D-mannose (10 mM)	Granulocyte interaction			
		Association	Ingestion	Metabolic activation	Killing[a]
(diagram)	Absent	–	–	–	–
	Present	–	–	–	–
(diagram)	Absent	++	(+)	+	–
	Present	–	–	–	–
(diagram) IgG1	Absent	++++	+++	++++	++++
	Present	–	–	–	N.D.
(diagram) IgM	Absent	N.D.	N.D.	N.D.	++++
(diagram) IgG1	Absent	++	++	++	N.D.
	Present	(++)	(++)	(++)	N.D.
(diagram) IgG1	Absent	++	(+)	+	N.D.
(diagram) IgG1 / IgG1	Absent	+++	++	+++	N.D.

← type 1 pili

← capsule

[a]: Killing measured in the presence of complement;
N.D.: Not determined;
The reactions were graded: ++++ very strong; +++ strong; ++ good; + slight; (+) very slight; – no reaction.

In vitro Characteristics of the Monoclonal K13 Polysaccharide Antibodies

All IgM and several IgG monoclonal antibodies against the K13 Ps rapidly agglutinated encapsulated *E. coli* K13 at room temperature. The agglutination caused by the IgG antibodies may be due to the polysaccharide antigen being composed of repeating units possibly facilitating cross-binding and agglutination. In neonatal rats, we previously demonstrated passive protection against invasive disease from *E. coli* by capsular milk antibodies (10). It is possible that the presence of agglutinating antibodies in the mucus layer lining the intestinal mucosa may contribute to protection against infection.

Monoclonal K13 Ps antibodies increased the association of *E. coli* K13 with human polymorphonuclear leukocytes (PMNL) (11). Furthermore, the ingestion of bacteria and the metabolic activation of the PMNL were increased in the presence of K13 Ps antibodies (Table 2).

The *E. coli* K13 were type 1 piliated, as demonstrated by mannose-sensitive hemagglutination of guinea pig cells (12) and bacterial agglutination by monoclonal antibodies against type 1 pili (6, 11). Type 1 piliated bacteria are more hydrophobic than non-piliated bacteria (13), they associate better with PMNL and trigger chemiluminescence in the absence of bacterial ingestion (11, 14). The addition of 10 mM D-mannose completely abolished the opsonizing capacity of the capsular antibodies (Table 2). The findings shown in Table 2 illustrate a striking influence of pili on bacterial association with PMNL. As no complement was present in the reaction mixture, one may speculate that the interaction was mediated by Fc receptors and mannose-containing receptors on the PMNL (11). In the presence of mannose, the first step of the interaction, involving the binding of pili to mannose residues on the PMNL surface, was prevented. This apparently blocked the binding of the K13 Ps antibodies coating the bacteria to the Fc receptors on the PMNL. Man-

nose did not directly affect Fc binding, as demonstrated by the negligible effect of mannose on the opsonization by anti-K13 Ps on the non-piliated bacteria (Table 2).

D-mannoside or other "receptor analogues" may be used to counteract the invasion of potentially pathogenic microorganisms by preventing binding to specific receptors on epithelial cells. A possible harmful consequence of soluble "receptor analogues" is suggested by the present findings. If PMNL or other phagocytes utilize similar receptors as the epithelial cells for binding, e.g. of bacteria, "receptor analogues" bound to the bacterial surface may be harmful once the bacteria reach the bloodstream. The killing of the bacteria was mediated by IgG1 as well as IgM anti-K13 antibodies (Table 2). These results support previous findings of a protective capacity of capsular antibodies *in vivo* (15, 6).

Monoclonal IgG1 anti-idiotype (anti-anti-K13 Ps) itself had no effect on the bacteria-PMNL interaction. The addition of anti-idiotype to bacteria preopsonized with an-

ti-K13 Ps antibodies, however, decreased the association and ingestion as well as the metabolic activation (Table 2). One may speculate that the anti-idiotypic antibodies were capable of competing with the bacteria binding to the anti-K13 Ps antibodies (16).

Protective Capacity of Monoclonal Antibodies Against the K13 Polysaccharide

The protective capacity of the monoclonal K13 Ps antibodies has been studied previously. Adult CBA mice were challenged intraperitoneally with live *E. coli* 06:K13:H1 five hours after intraperitoneal injections of polyclonal rabbit anti-O6 antibodies or IgM monoclonal anti-K13 Ps antibodies (150C8). The monoclonal anti-K13 Ps antibodies protected the mice from death as well as the hyperimmune rabbit O6 antiserum (17).

Monoclonal IgG and IgM anti-K13 antibodies given intraperitoneally to Sprague-Dawley rats prior to infection with live *E. coli* O6:K13:H1 protected the rats from ascending pyelonephritis (18).

Neonatal Administration of Idiotype or Anti-idiotype Primes for Protection Against E. coli K13 Infection

Monoclonal anti-idiotypic antibodies were prepared as previously described (16). One hybridoma, 150C8, an IgM anti-K13 Ps, was injected with complete and incomplete Freund's adjuvant into A/HeJ mice. The spleen cells were fused with SP2/0 cells as previously described (6). Clones producing anti-idiotype were selected by ELISA, coating the microtiter cells with the K13 Ps, followed by a 1:50,000 dilution of 150C8. One clone, 5868C, an IgG1, was selected for further study using ammonium sulphate precipitated ascites fluid produced in CFA$_1$ mice. The specificity of the idiotype/anti-idiotype interaction was tested by hemagglutination inhibition (6, 16). Inhibition by the K13 Ps was 12 logs more effective than inhibition by the serologically related K20 and K23 polysaccharides. This finding suggests that the 5868C reacts with the antigen combining site of the 150C8.

The injection of nanogram doses of the 5868C anti-idiotype into adult Balb/c mice induced the formation of anti-K13 Ps antibodies, although great individual variations were observed (*Söderström* and *Stein*, unpublished observations). Intraperitoneal injection of 100 µg anti-idiotype initially induced the formation of the non-K13 Ps-binding 150C8 idiotype, but after seven to 14 days the idiotype level was suppressed (*Söderström* and *Stein*, unpublished observations). These results were obtained using an ELISA assay in which the inhibition of 150C8 reactivity with enzyme-labeled 5868C was measured.

Neonatal Balb/c mice were immunized intraperitoneally with K13 Ps, idiotype or anti-idiotype, within a few hours after birth. The mice were then given intraperitoneal injections of formalin-killed *E. coli* K13 or K13 Ps at either four or 12 weeks of age (Figure 1).

Mice injected at birth with 1 µg of 150C8 or 50 ng of 5868C and immunized with killed *E. coli* K13 at four weeks of age were protected from death following infection with 20 LD$_{50}$ *E. coli* K13 at five weeks of age. Mice given K13 Ps at birth were not protected (16).

When the mice were given the K13 Ps at four weeks of age, mice primed both with idiotype or with anti-idiotype were again protected. Only the mice given anti-idiotype, however, were protected when the K13 Ps injection was delayed until 12 weeks of age followed by challenge with 50 LD$_{50}$ live *E. coli* K13 at 13 weeks of age (16).

These results show that the monoclonal anti-idiotype as well as the anti-K13 idiotype may prime neonatal mice for protection against lethal infection with live *E. coli* K13 following immunization with antigen. The administration of a protective idiotype may possibly serve the dual function of passive protection and priming for protective antibodies upon exposure to antigen. The administration of anti-idiotype may strongly influence the immune network and possibly cause long-standing effects on the immune repertoire.
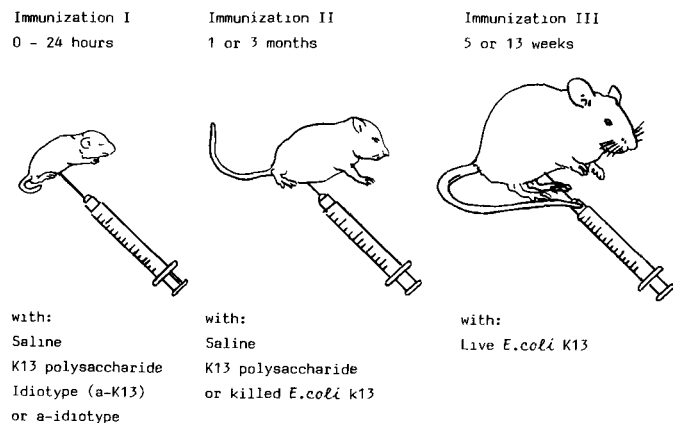
Figure 1: Immunization schedule for neonatal priming of Balb/c mice followed by injections of *Escherichia coli* K13 polysaccharide or formalin-killed *Escherichia coli* K13 and challenge with live *Escherichia coli* K13.

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Discussion

Dr. Siegel: I wonder, did you test K13 polysaccharide in a wide range of doses to see if a lower dose could have more protection?

Dr. Söderström: We have only tried the 2.5 μg dose so far, but we are at present carrying out experiments using higher and lower polysaccharide doses and also low doses of idiotypic antibodies. The 1 μg dose was used to obtain a protective level of idiotypic antibodies neonatally.

Dr. Gotoff: Have you done any experiments to test your hypothesis that the anti-idiotypic antibody is reacting with the B cells either by *in vitro* or *in vivo* experiments?

Dr. Söderström: No, my discussion on that point is based on findings in other idiotype systems with characteristics shared with the K13 system.

Dr. Young: Your obvious area for study is K1 immunization; does it work in this system?

Dr. Söderström: We have also worked with the K1 system and we have been lucky to obtain monoclonal antibodies which seem to react either with terminal sialic acid or with the poly-sialic acid. We will try to make anti-idiotypic antibodies against these antibodies to see if priming with these antibodies will affect the polysaccharide immune response upon antigen administration. This may be important since some sialic acid antibodies may bind to structures in the tissues.

Dr. Kaufmann: Do you have any idea how to overcome the failure to induce immunity in adult mice with anti-idiotypic antibodies by using adjuvants or cyclophosphamide or something like that, which has been used in other systems?

Dr. Söderström: We avoided using adjuvant in these studies. We tried, however, to induce antibodies by anti-idiotypic stimulation of adult mice. In some of these experiments we found that a low dose of anti-idiotypic antibodies administered i.p. without subsequent antigen administration increased idiotype (anti-K13) formation. The individual variations found in these animals may reflect the fact that they have different antigenic backgrounds. Lack of correlation between antibody levels and protection may be explained by different proportions of protective idiotypes among antibodies with a similar paratope.

Dr. Drews: You had that scheme showing priming with different doses of the anti-idiotypic antibody and the subsequent booster with K13. Could one get the same effect that you got with your low dose priming and the later booster with K13 if one used the low dose priming with anti-idiotypic antibody and boostered with anti-idiotypic antibody again?

Dr. Söderström: We have done preliminary experiments along those lines and have variable results. Maybe in the K13 system, the effect of idiotype-anti-idiotype administration may only occur neonatally or before environmental antigens have had a profound influence on the immune system.

Dr. Robbins: Can you predict what will happen if you inject a conjugate with K13 polysaccharide conjugated to the anti-idiotypic antibody in the neonatal mouse?

Dr. Söderström: Dr. Robbins, if you make that conjugate, we will find out.

Dr. Robbins: Do you think a monovalent fragment would induce the type of priming, or do you think the anti-idiotypic antibody is merely reacting with the surface immunoglobulin, forming clumps, patching and initiating the cell to undergo some maturation process?

Dr. Söderström: We do not know the mechanisms.