11 Cell adhesion molecules in enteropathic processes

Hill S M., Milla P J., Caseseca T., Mirakian R.

Dept. of Child Health, Institute of Child Health and Dept. of Immunology University College & Middlesex School of Medicine.

The immune response is successfully activated when cells expressing HLA class II molecules present antigen to T lymphocytes, and specific "accessory" molecules are also expressed on the surfaces of the cells involved to allow effective cell adhesion. Two such molecules are intercellular adhesion molecule 1 (ICAM-1) detected on antigen presenting cells which acts as a ligand for lymphocyte function associated antigen 1 (LFA-1) found on leucocytes. In Coeliac Disease (CD) and autoimmune enteropathy (AIE) aberrant class II/DR expression occurs on jejunal crypt enterocytes. We have previously suggested that in AIE altered antigen presentation initiates and/or perpetuates the enteropathic process. We now investigate whether altered adhesion molecule expression plays a role in this process. Jejunal mucosa from 7 children with AIE, 5 with CD, and 5 controls with histologically normal mucosa and normal DR expression was compared. Cryostat sections were stained by an indirect immunofluorescence technique using monoclonal antibodies to ICAM-1, LFA-1, and HLA-DR. In both CD and AIE aberrant DR expression by crypt enterocytes was present. ICAM-1 was expressed by crypt enterocytes in all with AIE and LFA-1 in 3/7. Both ICAM-1 and LFA-1 were negative in CD and control crypt cells and in all villous enterocytes. Throughout the lamina propria of all biopsies LFA-1 and ICAM-1 +ve cells were detected but in greater numbers with more intense staining in AIE. Thus in AIE but not CD aberrant DR expression correlates with enhanced and inappropriate epithelial cell adhesion molecule expression and this may facilitate autoantigen presentation by these enterocytes and so perpetuate the enteropathic process.

12 Congenital chloridorrhoea (CCD) without diarrhoea!

Milla P J., Bisset W M., Sanderson I., Jenkins H R.

Hospitals for Sick Children and Institute of Child Health, London.

Congenital chloridorrhoea is an inherited life threatening disorder usually with profuse watery diarrhoea which starts in utero. The diarrhoea is characterised by large volumes of chloride rich watery stools due to defective anion exchange transporters in the ileum and colon. We have investigated Cl^- transport in the colon using non-equilibrium rectal dialysis in children with CCD and controls. 2 Caucasians had classic CCD with diarrhoea from birth (GpA.) but 4, 3 from the Arabian Gulf and 1 from Nigeria presented with marked hypochloreamia (21-77mmol/l), hypokalaemia, severe failure to thrive and unusually passed formed stools (GpB). Because of this feature diagnosis was delayed in B and in 2/4 an erroneous diagnosis of Bartters syndrome was made. All four in B had marked secondary hyperaldosteronism plasma aldosterone 590 - >3300 pmol/l nr 96-946; plasma renin 4300 to 9530 A1/L/hr nr 110-2610; rectal potential differences (PD) 82-100 mv nr 25-40. Following rehydration and correction of electrolyte deficit in B profuse watery diarrhoea developed with stool chlorides of 124-153mmol/l which exceeded the sum of Na+ and K+ $conc^2$. In A using a dialysate of 130 mM Cl^-, Cl^- secretion of -70 and -32nmol/min/cm^2 was seen compared to absorption in controls (n=17) + 195+55 mean + 1SD and B+26--+69. In 1 of B the effects of varying the Cl^- concentration of the incubation solution on Cl^- fluxes was determined. 130mM +69, 90mM-69, 60 mM-107, 30 mM-223 nmol/min/cm^2. These data suggest that Cl^- is moving passively down a chemical gradient whereas in A active secretion occurs. As diarrhoea in CCD is due to the osmotic effect of luminal Cl^- when Cl^- depletion is present it may not occur. We speculate that in CCD different transporter defects may occur and these these may determine the presenting features.

13 THE CYTOKINES INTERFERON-γ (IFN-γ) AND INTERLEUKIN 4 (IL-4) PROMOTE TRANSCYTOSIS OF SECRETORY IgA (SIgA) IN HUMAN EPITHELIAL CELLS IN VITRO.

Zina Moldoveanu, Serem Freier, John Phillips, Jiri Mestecky.

Department of Microbiology and Medicine, University of Alabama at Birmingham, and the Pediatric Research Laboratory, Shaare Zedek Medical Center, Jerusalem. We have previously demonstrated that the HT-29 human colon carcinoma cell line expresses secretory component (SC), and that culture of these cells with IL-4 and with IFN-γ considerably augments the expression of SC in these cells. Our objective here was to ascertain if this is associated with increased transcytosis of SIgA across the enterocyte. Transparent Biopore membrane inserts were coated with human placental collagen matrix. The coated inserts were placed in 6 well cell cluster culture plates, 36 mm in diameter. On the following day, HT-29 cells maintained in RPMI 1640 were seeded in the inserts in a concentration of 10^7 cells/ml. RPMI 1640 only was placed in the outer well. Twenty four hours later the cells formed a macroscopic monolayer on the filter. INF-γ and IL-4 were added in a concentration of 100u/ml each. Forty eight hours later, ^{125}I-labelled monomeric IgA (mIgA) (control) and polymeric IgA (pIgA) (test) were added to the inserts. After 24 h, total and SC-associated radioactivity were measured in the fluid of the outer wells. It was found that SC-associated radioactivity was 3x higher in the pIgA wells than in the mIgA wells. It was also 3x higher when compared to filters coated with the CaCo 2 human carcinoma cell line, shown by us not to express SC. CONCLUSION: IFN-γ and IL-4 promote SIgA transcytosis in the HT-29 cell line. Supported by NIH grants AI 18745, AI-10854, DK-28537 and GIF grant T-79-063.2/88.

14 DESCRIPTION OF A PUTATIVE ENTEROTOXIN PRODUCED BY ENTEROAGGREGATIVE ESCHERICHIA COLI (EAggEC).

S.J.Savarino, A.Fasano, D.C.Robertson, M.M.Levine

Univ. Maryland Sch. Medicine, Baltimore, Maryland USA

EAggEC have been associated with persistent diarrhea in children, yet little is known about virulence factors. Aim. We assayed for enterotoxic activity in culture filtrates(CF) of EAggEC strains. Methods. CF from 17-2 (a prototype Chilean EAggEC), 8 other EAggEC strains and E. coli HB101 were added to stripped rabbit ileal mucosa(RIM) mounted in Ussing chambers(UCs). CFs were also tested by E. coli STa ELISA and suckling mouse assay (SMA). EAggEC colonies were hybridized under low stringency to STh and STp DNA probes. Results. Addition of 17-2 CF to the RIM in UCs caused a greater rise in short circuit current (ΔIsc=55.2 ± 5.3 uAmp/cm2)(Mean ± SE) than that seen in HB101 control (ΔIsc=16.8 ± 3.0 uAmp/cm2) ($p < 0.001$). This activity on Isc was 1)65% stable after heating (15 m at 65°C); 2)localized to the 2-5 kDa MW size range; and 3) reproduced with CF from HB101 transformed with 17-2 plasmid. Compared to ΔIsc response to 8-Br-cGMP on negative control tissue (ΔIsc=123.4 ± 7.9 uAmp/cm2), the ΔIsc to 8-Br-cGMP added to tissue pretreated with 17-2 CF (87.55 ± 8.2 uAmp/cm2)was significantly less ($p < 0.01$), while the additive effect of 17-2 CF and 8-Br-cGMP (ΔIsc=142.8 ± 9.1 uAmp/cm2) was not significantly different ($p > 0.05$). <10 kDa CF fractions from 6/8 other EAggEC strains screened in UCs gave ΔIsc responses similar to 17-2. The 17-2 CF activity was 1)non-neutralizable with polyclonal anti-STa in UCs; 2)non-reactive by STa ELISA; and 3)negative in SMA. Also, EAggEC colonies did not hybridize with STh or STp DNA probes. Conclusions. EAggEC strain 17-2 produces an extracellular, low MW, partially heat stable moiety which gives an electrical response in vitro consistent with an enterotoxic effect. This putative EAggEC heat stable enterotoxin (EAST) appears to be plasmid-mediated, not genetically or immunologically cross-reactive with E. coli STa, and may act via cGMP as intracellular mediator.

15 PRODUCTION OF A SECOND DIARRHEAGENIC FACTOR BY V. CHOLERAE.

A. Fasano, B. Baudry, D.W. Pumplin, B.D. Tall, J.M. Ketley, J. B. Kaper. U. MD Sch. Med., Baltimore, MD 21201, U.S.A.

In the last decade a great effort has been made to produce an efficient vaccine against cholera. When fed to volunteers, some genetically engineered mutants lacking the cholera toxin(CT)genes(CVD 101)induced mild diarrhea(1), while others(395N1)did not(2). Aim of the present study was to evaluate whether toxic factors other than CT are involved in the pathogenesis of cholera. Methods: crude culture supernatants from V. cholerae 395 and from two similarly constructed CT- mutants(CVD101 and 395N1) were added to rabbit intestine stripped of serosal and muscular layers and mounted in Ussing chambers. At the end of the experiments, tissues were processed for electron microscopy. Results: a)395 and CVD101 supernatants added to the mucosa of small intestine gave a significant increase in tissue conductance(Gt) peaking after 2hrs. This increase caused an early increase in short circuit current(Isc)that was not related to CT activity. No change in Gt was observed when 395 supernatant was added to the caecal tissue; b)395N1 did not increase Gt for 100 min. when added to the small intestine; c)preliminary freeze-fracture data showed that tight-junctions(tj)became less complex, having fewer intersections, in tissues exposed to 395 or CVD101 supernatants, compared to those exposed to medium alone. Conclusions: 1) 395 and CVD101 supernatants induced a significant increase in Gt in rabbit small intestine, while 395N1(non diarrheagenic CT- mutant) did not. Exposing caecum to 395 did not alter Gt; 2)the Gt increase was associated with morphological changes of tj; 3)this factor may be responsible for the residual diarrhea observed in some CT- mutants.

1. Infect. Immun. 56, 161-168(1988); 2. J. Exp. Med. 168, 1487-1492(1988).

16 IN SITU HYBRIDIZATION FOR DETECTION OF HEPATITIS B VIRUS GENOMES IN LIVER TISSUE OF CHRONIC INFECTED CHILDREN

S. Wirth, A. Hueter, K.M. Keller, W. Baumann, B. Zabel

Children's Hospital, Johannes Gutenberg-University, D-6500 Mainz

Detection of hepatitis B virus (HBV)-DNA in the liver of chronic infected patients is presently the most sensitive marker of viral replication and infectivity. In situ hybridization (ISH) allows the direct visualization of HBV infected liver cells and distribution of the viral sequences. This study was done to establish ISH and correlate the findings with conventional markers for HBV infection.

Methods. Liver biopsies of 50 patients (28 ♂, 22 ♀) aged 0.5-20 years (mean 10.3) with various histological diagnoses were tested by ISH. The HBV-DNA probe was labeled by nick translation with ^{35}S-CTP to a specific activity of $3\text{-}5 \times 10^8$ cpm/µg DNA.

Results. HBV-DNA/mRNA could be demonstrated in 38 patients, 12 were negative. Distribution of the grains was homogenous, inhomogenous with focal patches and focal. 33/38 children with HBeAg and 4/11 with anti-HBe were positive by ISH, 5/38 with HBeAg and 7/11 with anti-HBe remained negative. 17/23 HBsAg carriers with positive HBV-DNA/mRNA by ISH were positive for HBcAg in the liver and 31/36 had free HBV-DNA in Southern blot hybridization.

Conclusions. Our results indicate that hepatitis B viral genomes can reliably be detected by in situ hybridization. Although there is a good correlation to other HBV markers (HBeAg, HBcAg and Southern blot hybridization) ISH may represent a more sensitive method to prove viral replication and infectivity.