5

THE IMMUNE RESPONSE TO SHIGELLA FLEXNERI 2A INFECTION IN MONKEYS. G. Dinari, T.L. Hale, S.B. Formal. Pediat. GI Unit, Beilinson Hospital, Israel, and Dept. of Enteric Infections, Walter Reed Army Institute of Research, Washington DC, USA.

Shigella infections are characterized by invasion of epithelial surfaces. A 140 megadalton plasmid was shown to be responsible for the expression of virulence. This plasmid encodes several outer membrane proteins, which are absent in the plasmid free avirulent organisms. As invasion does not occur in immune subjects, it seemed of interest to study the immune response to the plasmid coded proteins (PCP), and to compare it with the response to the somatic antigen, lipopolysaccharide (LPS).

Five monkeys were infected by a S. flexneri 2a strain. Blood was drawn at 0,7,14,30 days post infection. Rectal biopsies were taken at the same time, and cultured for 24 hrs. Blood and culture media immunoglobulins were measured and characterized by ELISA and immunoblotting techniques.

PCP were found to be of major importance in the immune response IgG serum antibody to PCP reached a higher titer than that to LPS, and persisted longer. A similar IgA and IgG response was found in the intestine. Four PCP proteins were recognized by most animals, as determined by immunoblotting.

This new information suggests that the PCP serve a major role in the host response to infection, and deserve further studies to clarify their role in the pathogenesis of shigellosis.

6

IMPAIRMENT OF MUCOSAL HYPERPLASIA FOLLOWING MASSIVE SMALL BOWEL RESECTION BY ESSENTIAL FATTY ACID (EFA) DEFICIENCY by J.A. Vanderhoof and M.H. Hart, University of Nebraska Medical Center and Swanson Center for Nutrition, Inc., Omaha, Nebraska, USA.

Previous studies have shown that dietary long chain triglycerides stimulate mucosal hyperplasia following massive small bowel resection. In these studies, however, mucosal hyperplasia in "control" animals may have actually been inhibited by short term EFA deficiency. To evaluate this possibility 24 Sprague-Dawley rats were subjected to 60% proximal jejunoileal resection and subsequently pair-fed isocaloric diets containing either linoleic (control) or palmitic (EFA deficient) acid (5% fat by weight). Two weeks postoperatively, mucosal protein (mg/cm bowel + SEM) was determined in the remaining bowel:

	Duodenum/Jejunum	Proximal Ileum	Distal Ileum
Control	4.83±0.37	6.02±0.61	4.86±0.57
EFA Deficient	3.02±0.25	3.51±0.22	3.24±0.34

Mucosal protein levels were decreased in EFA deficient resected animals in all segments in remaining bowel ($p<.05$). Mucosal DNA levels paralleled these changes. A subsequent study demonstrated rapid reversal of impairment of mucosal hyperplasia following reinstitution of EFA containing diets. Hepatic triene:tetraene ratios confirmed EFA deficiency in the palmitic acid group. EFA deficiency impaired mucosal adaptation following resection. This effect was reversible, and may explain stimulatory effects of long chain triglycerides on mucosal hyperplasia.

7

DIFFERING IMMUNE MECHANISMS TRIGGERING AUTOIMMUNITY IN PRIMARY SCLEROSING CHOLANGITIS (PSC) AND AUTOIMMUNE CHRONIC ACTIVE HEPATITIS (aCAH) OF CHILDHOOD G.Mieli-Vergani,A.Lobo-Yeo,AP Mowat, D.Vergani.Depts. Child Health & Immunology, King's College Hospital London.

PSC histological features may resemble those of aCAH and increased autoantibody and IgG levels are found in both conditions. PSC, however, is less responsive to immunesuppressants. To investigate whether this derives from differences in regulatory and/or effector immune mechanisms we have studied 7 children with PSC, 14 with aCAH and 26 healthy children as controls. Lymphocyte cytotoxicity values to autologous hepatocytes were similarly elevated in 5 children with PSC (mean±SD, 50±9%) and in 7 with aCAH (53±16%) studied. In contrast, T suppressor number and Con-A induced function were normal in patients with PSC (23.5±3.1% and 50.8±5.3%), but significantly decreased in children with aCAH (15.8±4.7% and 7.6±6.5%), when compared to controls (24.2±5.4%, $p<0.02$ and 51.7±4.0, $p<0.01$ Rank Test). In 7 PSC patients percentages of activated T cells expressing HLA-DR (4.4±1.2%) or IL2r (0.2±0.3%) were similar to controls (2.92±0.3% and 0.14± 0.2%) while significantly increased numbers of both were found in 14 aCAH patients (6.3±2.7, $p<0.05$ and 18.0±3.2 $p<0.001$). Our data suggest that liver damaging effector mechanisms are similar in PSC and aCAH but factors triggering autoimmunity differ. While in aCAH autoimmunity probably derives from defective T-dependent immune regulation, in PSC it could result from B lymphocyte polyclonal activation bypassing T cells.

8

THE INFLUENCE OF HLA STATUS IN DETERMINING SUSCEPTIBILITY TO BILIARY ATRESIA IN PATIENTS WITH & WITHOUT CONGENITAL ANOMALIES IN OTHER SYSTEMS. Donaldson PT, Silveira TR,Mieli-Vergani G,Howard ER,Mowat AP.Depts. Liver Unit,Child Health & Surgery.King's Coll.Hosp.

Biliary atresia is characterised by complete obstruction of the extrahepatic bile ducts.Its aetiology is unknown.It may be a congenital structural abnormality or an obliterative inflammation of the bile ducts. To assess whether genes of the HLA region confer susceptibility to biliaryatresia, we have performed in 49 controls & 55 patients HLA typing for 42 Class 1 (A & B) antigens & for 12 Class II (DR) antigens in 28 patients. 8 (15%) had major abnormalities outside the hepatobiliary system. The frequency of Class II antigens was similar in controls & patients. Significant differences occur only with the Class I antigen HLA-A10 and B12. HLA-10 was absent in all patients and present in 6 controls. ($p<0.01.X^2$) B12 was present in 11 controls and 24 patients ($p<0.02$) occurring in 23 of 49 ($p<0.01$ compared with controls) without congenital anomalies in other systems and in only 1 of 8 with such malformations. The absence of HLA-10 suggests a protective action of this antigen. The increased frequency of B12 in patients without other congenital malformations may indicate that they may have genetically-determined susceptibility to biliary atresia, possibly through impaired response to exogenous antigens. It may be helpful in investigating possible aetiological factors in biliary atresia to consider two aetio-pathogenic groups,ie those with or without other abnormalities.

9

BILIARY ATRESIA AND REOVIRUS TYPE-3 INFECTION R.H.J. Houwen, R.J.A. Diepersloot and Ph.H. Rothbarth Dept. of Pediatrics, University Hospital, Groningen Dept. of Virology, University Hospital, Rotterdam

In a recent American study it was found that 17 of 25 patients with extrahepatic biliary atresia had IgG antibodies to reovirus type-3 as compared with only 3 of 37 age matched controls. However, an European group found antibodies to reovirus type-3 in only 13 of 28 patients and in 15 of 30 controls. So it was concluded that reovirus is not an important cause of biliary atresia in Europe.

We have performed an identical study using a similar serologic method. IgG antibodies to reovirus type-3 were detected in 16 of 23 neonates with extrahepatic biliary atresia (70%), while only 14 of 35 age-matched controls were positive (40%) ($p < 0.05$).

We also tested maternal blood of 16 children with biliary atresia. Fourteen mothers had IgG antibodies against reovirus (88%), while 5 of 7 controls (70%) were positive. To investigate the occurrence of a recent infection in the mothers, we tested these maternal sera for IgM antibodies. Three out of sixteen mothers of infants with biliary atresia had high titres of IgM antibodies to reovirus. None of seven controls was positive.

These results suggest a major role for reovirus type-3 infection in the causation of extrahepatic biliary atresia. In addition, infection of the mother could be important in the pathogenesis of biliary atresia in their children.

10

RELATIONSHIP BETWEEN GLUCOSE AND SODIUM IN ORAL REHYDRATION SOLUTIONS (ORS); STUDIES IN A MODEL OF SECRETORY DIARRHOEA. E.J.Elliott, J.A.Walker-Smith, M.J.G.Farthing Departments of Child Health and Gastroenterology St.Bartholomew's Hospital, London.

Glucose sodium co-transport remains intact in enterotoxin - mediated diarrhoea, hence the rationale for rehydration with oral glucose electrolyte solutions. Using a technique of in situ perfusion in whole rat small intestine after exposure to cholera toxin (75μg for 2 hours) we evaluated the ability of seven different ORS (Glucose 111 or 202 m mol/l, sodium 35-90 m mol/l, osmolality 270-320 mOsm/kg, ratio of glucose: sodium concentration 1.2-5.8), to reverse net water secretion to absorption. Water absorption (+) in μl/min/g of dry wt intestine occurred with all ORS but was greater from ORS with a glucose: sodium ratio of 1.9 than from ORS with ratios of 1.2 and 5.8 (+ 98.6 ± 16 vs + 37.8 ± 8 vs 2.2 ± 7 respectively; $p < 0.01$). Hypotonic ORS (270 mOsm/kg were superior in promoting water absorption than ORS with osmalities of 312 and 330 mOsm/kg. Water absorption was less from the two ORS with glucose 202 m mol/l. (+ 2.2 ± 7 and 19.4 ± 4) than from the five ORS with glucose 111 m mol/l. (range + 37.8 ± 8 to + 101.9 ± 12; $p < 0.01$). ORS with 60 m mol/l of sodium were superior to those with 35 or 90 m mol/l. Efficacy of ORS in this model of secretory diarrhoea depends not only on glucose and sodium concentration but on the glucose: sodium ratio and osmolality. These studies may guide development of new, more effective ORS.