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EXPRESSION OF CLASS I & CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX)MHC) ANTIGENS ON HEPATOCYTES ISOLATED FROM CHILDREN WITH LIVER DISEASE.

A Lobo-Yeo,G.Mieli-Vergani,AP Mowat,D.Vergani.Depts. Child Health & Immunology,King's Coll.Hosp.London.

Immune reactions to hepatocyte membrane antigen has been suggested to play an important role in liver damage in several conditions.Antigens on the surface of cells are recognised in association with glycoproteins coded for MHC. In this study we have investigated expression of MHC class I & II antigens on isolated hepatocytes from 24 children with liver disease: 2 autoimmune chronic active hepatitis, 4 primary sclerosing cholangitis, 5 alpha-1 antitrypsin deficiency, 3 post-liver transplant, 3 hepatitis B related chronic liver disease, 7 minor histological changes.Hepatocytes were isolated from liver biopsies by mechanical disruption.Class I(HLA-A,B & C) antigens were detected by W6/32 monoclonal antibody(Mab) and Class II(HLA-DR) antigens by L243 Mab. Class I antigens were present on isolated hepatocytes in all cases while Class II were never detected. Positive stain for Class II was seen on Kuppfer cells. Expression of HLA-DR antigens on hepatocytes could be induced by a 72hr.incubation with 200 U of recombinant gamma interferon. These data suggest that immune recognition of hepatocyte membrane antigens may not be class II restricted. Alternatively, the HLA-DR positive Kuppfer cells might act as liver antigens presenting cells.

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IgG SUBCLASSES IN AUTOIMMUNE CHRONIC ACTIVE HEPATITIS (aCAH) OF CHILDHOOD.

A Lobo-Yeo, G Senaldi,G Mieli-Vergani, AP Mowat, D Vergani
Depts.Immunology & Child Health,King's Coll.Hosp.London

An increase in IgG levels is characteristic of aCAH but it is not known whether such increase affects all subclasses. Indeed, we have recently shown that the anti-liver/kidney microsomal antibody in aCAH is restricted to IgG1. In this study we have measured IgG subclasses using an ELISA technique. In 57 serum samples from 15 children with aCAH [11 female,age range 3-18yrs]. In all untreated cases the total IgG levels were increased and all subclasses were also increased when compared to 97 age-matched controls.[Patients:IgG1 mean ± SD = 10.4 ± 2.63,IgG2 = 4.8 ± 1.24, IgG3 = 2.8 ± 0.63,IgG4 1.3 ± 0.4g/l. Controls;IgG1 = 6.56 ± 1.87,IgG2 = 2.47 ± 0.76, IgG3 = 0.86 ± 0.31,IgG4=0.312 ± 0.22g/l, the differences being significant for IgG2,$p<0.05$;IgG3, $p < 0.01$; IgG4,$p<0.01$].Moreover,percentages of IgG3 and IgG4 were higher in patients when compared to age-matched controls [patients; IgG3 14.5%,IgG4 7.7%: Controls; 8.6% and 3.6%respectively]. After effective immunosuppressive therapy,the total concentration of IgG was reduced, but the percentage distribution of the subclasses remained altered compared to controls.[IgG1, IgG2,IgG3,IgG4 being 56,23,11.5 and 9.5% in patients, 63,25,8,6 & 3.5% in controls]. These data show that children with aCAH have an overall absolute increase of IgG subclasses with a preferential increment of IgG3 & IgG4 which is not related to treatment or disease activity.

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ANTINUCLEAR ANTIBODIES(ANA) ARE IgG1 SUBCLASS RESTRICTED IN AUTOIMMUNE CHRONIC ACTIVE HEPATITIS(aCAH) BUT NOT IN PRIMARY SCLEROSING CHOLANGITIS(PSC) OF CHILDHOOD.

M.Peakman,G Mieli-Vergani,AP Mowat, D.Vergani.Dept.Immunol & Child Health,King's Coll.Hosp. London.

Children with aCAH and those with PSC have similarly high levels of IgG, non organ and liver specific autoantibody & increased lymphocyte cytotoxicity to autologous hepatocytes.However,they differ in several immunological parameters.Only in aCAH there is decreased T suppressor cell number & function and increased circulating activated T helper cells,suggesting different mechanisms for the emergence of autoimmunity.Antinuclear antibody(ANA) is characteristic of both PSC and a sub-group of aCAH.The aim of this study was to characterise ANA in these 2 conditions. Using an indirect immunofluorescence technique and monoclonal antibodies to IgG subclasses,we have determined the immunoglobulin class and IgG subclass of ANA in 5 patients with aCAH[3 female,median age 18yrs, range 15-19yrs]& in 8 with PSC[4 female,median age 8.5yrs,range 3-14yrs].Patients with aCAH had titre of ANA between 1:10 & 1:640 & in all of them ANA belonged to the IgG class and IgG1 subclass. In contrast,in children with PSC ANA(titres 1:10-1:2560) were only IgG in 6 and IgG and IgM in 2.Of 6 patients with ANA titres of more than 1:10,3 expressed ANA in 2 subclasses[IgG1 & IgG3 in 2, IgG1 & IgG4 in 1], and 1 patient expressed ANA in all 4 subclasses. These differences in ANA class & subclass between aCAH & PSC could derive from differences in the nature of the nuclear antigen or could indicate that ANA production results from the breakdown of different immunoregulatory mechanisms.

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LOW C4 HAEMOLYTIC FUNCTION IN AUTOIMMUNE CHRONIC ACTIVE HEPATITIS(aCAH)OF CHILDHOOD.

G Mieli-Vergani,AP Mowat, D.Vergani,G.Senaldi
Depts.Immunology and Child Health, King's College Hospital, London.

We have shown that children with aCAH have genetically determined low C4 levels but it is not known whether C4 function is also impaired. In the present study we measured haemolytic function of C4 and its concentration in 20 children with aCAH(15 female,median age 13½yrs,range 7-18yrs) and in 19 sex and age matched normal controls. C4 function was measured by a radial haemolytic assay,using C4 deficient guinea-pig serum, the concentration by laser nephelometry. A significant reduction of C4 levels was observed in patients [median 0.15g/l,range 0.04-0.485 g/l] compared to healthy children [0.263g/l, 0.153-0.520g/l,$p < 0.002$] confirming our previous data. C4 function in 16 normal children was 50% (25-100%) but in 3 was very low (4%, 4%,12%). In patients C4 function was 12%(0-67%), significantly reduced when compared to all controls.(41.5%, 4-100%,$p< 0.001$). There was no significant correlation between function and concentration values. Such discrepancy between function and concentration could be explained by the marked genetic polymorphism of C4. These data show that C4 function is impaired in aCAH. C4 has a key role in virus neutralization and impairment of its function might enable a virus to initiate the autoimmune process leading to CAH.

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DETECTION OF LYMPHOCYTES EXPRESSING HEPATITIS B VIRUS IN PERIPHERAL BLOOD FROM HBsAg POSITIVE AND NEGATIVE CHILDREN AND ADULTS BY "IN SITU" HYBRIDIZATION.

M. HADCHOUEL [1], C. PASQUINELLI [2], J. SCOTTO [1], R.N. HUGON [2], C. BRECHOT [2], O. BERNARD [1]. 1) INSERM U 56, Hôpital de Bicêtre, 94270 Bicêtre, France. 2) UREG, INSERM U 163, Institut Pasteur, Paris.

By using "in situ" hybridization methodology we have examined lymphocytes from peripheral blood from HBsAg positive and negative patients for the presence of HBV RNA. Mononuclear cells preparations were hybridized with a S35 or H3 labeled HBV specific DNA probe and exposed to autoradiographic emulsion.

42 subjects were studied (25 children, 17 adults). HBV RNA was present in the lymphocytes of 10/17 HBsAg+ HBeAg+, 8/18 HBsAg+ HBeAg-, 1/3 anti-HBs+ patients, 0/4 control subjects. The frequency of labelled cells varied from one patient to another from 1 to 10 %.

There is no difference between children and adult populations, and no correlation either the type of liver disease or presence of HBV DNA in the serum. These results demonstrate the presence of viral RNA In mononuclear cells and indicate that the DNA detected so far in human mononuclear cells is transcriptionnally active.

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NORMAL SERUM GAMMA-GLUTAMYL-TRANSPEPTIDASE (GGT) ACTIVITY IDENTIFIES A GROUP OF INFANTS WITH IDIOPATHIC COLESTASIS OF POOR PROGNOSIS

G. Maggiore, O. Bernard, M. Hadchouel, O. Alagille
Hépatologie Pédiatrique INSERM U 56, 94275 Bicetre, FRANCE

Serum GGT activity was assayed at diagnosis in 186 consecutive cholestatic infants aged 12 months or less followed for at least 1 year or until death. In 157 (biliary atresia 113, other extrahepatic obstructions 10, syndromic paucity of bile ducts 14, alpha-1-antitrypsin deficiency 6, other intrahepatic cholestasis of known origin 14) cholestasis could be classified in one of the known categories and serum GGT was raised in all (5-65 x N) but 1 with post-hemolytic conjugated hyperbilirubinemia in whom jaundice cleared within few months. In the 29 other, with intrahepatic cholestasis of unknown origin, results of serum GGT allowed to separate 2 groups: 17 infants with high GGT (2-13.7 x N) and 12 with normal GGT.In all but 1 in the first group jaundice resolved and results of clinical and laboratory investigation were normal at last control (2-42 months later. In all the children of the group 2 jaundice, pruritus and hepatosplenomegaly persisted: 6 died of liver failure and 3 others had ascitis and prothrombin time < 40%. Repated liver biopsies in 5 others showed progression of fibrosis; 1 child with liver failure underwent a successful liver transplantation.This group of cholestatic infants with normal serum GGT was further characterized as follows: parents were related in 6 instances and 6 infants had a sibling with a similar disease. Serum GGT remained normal througth the follow up period except in 1 in whom a transitory rise was associated with intrahepatic lithiasis wich cleared spontaneously. In conclusions these results suggest that in infants with cholestasis of unknown origin, persistently normal serum GammaGT helps identifying a group with progressive liver disease of poor prognosis which may correspond to an hereditary disease akin to Byler disease and may thus allow for early identification of those patients requiring liver trasplantation before patent signs of liver failure are present.