985 ONTOGENY OF B CELL FUNCTION IN HUMAN BONE MARROW. Toshifumi Hibi, Marcia A. Chan and Hans-Michael Dosch Research Institute, Hospital for Sick Children, Division of Immunology, Toronto, Ontario, Canada.

The development of the bone marrow B cell compartment was studied using marrow samples from adult and pediatric donors <1 yr old. The frequency, isotype commitment and secretory characteristics of B cells able to secrete immunoglobulin (Ig) was determined in limiting dilution cultures of 10-10,000 EBV infected and non-infected marrow cells. Ig secreting clones were detected by ELISA of supernates after 3-4 wk of culture. In striking contrast to adult marrow, pediatric marrow contained only small numbers of spontaneously Ig producing cells, most Ig producers were committed to IgM-production and most were EBV transformable:

MARROW SOURCE	EBV	Ig PRODUCERS per 100 B cells	ISOTYPE COMMITMENT (%) IgM	IgG	IgA
Adult	+	1.9	16	56	28
	-	1.4	1	78	26
Infant	+	1.2	81	10	9
	-	0.2	23	52	25

These differences approximate patterns of Ig-isotype expression in serum. However, the presence of similar numbers of B cells committed and able to produce IgG or IgA at a time when only IgG is expressed in serum suggests that isotype specific regulatory mechanisms, rather than 'B cell immaturity' direct the late expression of serum IgA. These regulatory mechanisms are reminiscent of IgA deficiency and may be bypassed in limiting dilution cultures.

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986 THE CELL SURFACE MOLECULE p24 IS HIGHLY CONSERVED ON VARIOUS TISSUES IN SEVERAL MAMMALIAN SPECIES. Richard D. Hockett, Jr., Jeffrey L. Platt, Jo Ellen Brown, John H. Kersey. University of Minnesota Medical School, Minneapolis, Minnesota.

The cell surface molecule p24 (human leukocyte differentiation antigen CD9) appears to be broadly distributed on primate (human and non-human) tissues by serologic analysis. The present study was designed to extend these serologic observations in lower vertebrate species and correlate serology by indirect immunofluorescence (IF) with structural analysis of p24 using lactoperoxidase ^{125}I labelled cells subjected to NP40 lysis, SDS-PAGE and autoradiography. Monoclonal antibody BA-2, which identifies an epitope of p24, reacted by IF with human platelets and fibroblasts, the MDCK (canine kidney epithelial), and BSC-1 (African green monkey kidney epithelial) cell lines, but not with bone marrow or mesonephrons of Rana pipiens. Immunochemical analysis identified p24 by identical migration in SDS-PAGE in each aforementioned cell type except Rana pipiens, which was not tested. BA-2 staining of normal canine kidney was compared with BA-2 staining of normal human kidney. Homologous reactivity was found with epithelium of Bowman's capsule and distal tubule and with arterial smooth muscle. In dog, but not human kidney, BA-2 reacted with mesangium. In human, but not dog, BA-2 identified endothelium.

Together, these results indicate that p24 is highly conserved in apparent molecular weight and in discrete cell types of mammalian species dating back at least 70 X 10^6 years.

987 HYDROCORTISONE (HC) INDUCED GREATER IN VITRO SUPPRESSION OF HUMAN POLYMORPHONUCLEAR GRANULOCYTE (PMN) AGGREGATION, AND OTHER PMN FUNCTION THAN METHYLPREDNISOLONE (MP) AND DEXAMETHASONE (DXM). F.Hodder, C.VandeVen, C.Wong, G.Bennetts, J.Katz, M.Cairo (Spon. by L.Gluck) Univ. Calif. Irvine, Child. Hosp. Orange Co. Orange, CA. 92668

Glucocorticoids are widely used in children as anti-inflammatory and cytoxic agents, controversially in acute respiratory distress syndrome and shock states. Their anti-inflammatory mechansim of action and the associated risk of infection is thought to be possibly secondary to PMN dysfunction. We comprehensively evaluated three anti-inflammatory glucocorticoids with differing potencies, investigating the effect and the dose response of HC, MP and DXM on in vitro human PMN function. PMNs were isolated to greater than 90% purity from peripheral blood layered on a dextran/ficoll-hypaque gradient. The cells were tested for FMLP (N-Formyl-l-methionyl-l-leucyl-l-phenylalanine) stimulated aggregation after incubation (60 min.) with HC, MP and DXM; CytoB/FMLP stimulated superoxide production and chemotaxic activity using the GAllin modification of the Boyden chamber technique with Cr^{51} PMNs stimulated by E.Coli endotoxin. PMN aggregation was significantly inhibited by all concentrations of HC, the least potent of the anti-inflammatory steroids and only by moderate and high concentrations of MP and DXM.

CONC	HC	P-Value	MP	P-Value	DXM	P-Value
CONTROL	PMN AGGREGATION 36.7±7.2	-	34.3±4.9	-	34.5±5.2	-
$8x10^{-3}$M	12.2±2.9	0.001	10.3±0.3	0.002	6.2±4.3	0.007
$1x10^{-4}$M	16.0±2.0	0.002	17.1±3.1	0.007	13.9±1.0	0.004
$5x10^{-4}$M	25.2±3.0	0.02	22.1±5.1	0.022	30.6±11.7	NS
$1x10^{-3}$M	24.2±2.8	0.015	26.0±6.7	NS	36.4±6.5	NS

Similar suppression of PMN superoxide and chemotaxis by HC was found at the highest concentration of $8x10^{-3}$M (Cont. 220±36 vs HC 17.6±11.4 nmoles/10^7 cells $p<.001$ and Cont. 22.4±3.8% vs HC 10.8±1.4% $p<.004$ respectively). Further studies of PMN bacterial killing and degranulation in vitro are in progress to be followed by in vivo studies using pharmacological doses.

● **988** INDUCTION OF COMPLEMENT-MEDIATED DESTRUCTION OF HOST CELLS BY BACTERIAL LIPOTEICHOIC ACID. Donna S. Hummell and Jerry A. Winkelstein. The Johns Hopkins University School of Medicine, Baltimore.

Lipoteichoic acids (LTA) released by gram positive bacteria can spontaneously bind to mammalian cell surfaces. Cells bearing LTA can activate either the classical or alternative pathways of complement (C') in heterologous sera. These observations suggest that the tissue damage which occurs during the course of a bacterial infection might in part be mediated by the host's C' system directed against its own cells bearing surface LTA.

A model system was established using erythrocytes (E) presensitized with pneumococcal LTA (LTA-E). When LTA-E from normal rats, normal humans, normal guinea pigs, a C2-deficient human and C4-deficient guinea pigs were each incubated in their autologous sera, there was significant C'-mediated lysis in vitro. The survival of ^{51}Cr-labelled autologous LTA-E in normal rats or cobra venom factor-treated rats (CoVF-rats) (<3% normal C3 levels) was also studied. Only 2.9% of autologous LTA-E remained in the circulation of normal rats at 90 min. In contrast, 31.2% of autologous LTA-E remained in the circulation of CoVF-rats. Intravascular hemolysis accounted for the clearance of LTA-E in the normal rats, while liver sequestration was responsible for clearance in the CoVF-rats. The survival of untreated autologous E in normal or CoVF-rats was normal (93.6% and 96.4% respectively).

The results demonstrate that LTA can render cells susceptible to damage by the host's own C' system, establishing this as a possible mechanism of tissue damage in natural infections.

† **989** IMPAIRED ANTIBODY RESPONSE TO BACTERIOPHAGE ØX 174 IN JUVENILE RHEUMATOID ARTHRITIS (JRA). Norman T. Ilowite, Hans D. Ochs, Ralph J. Wedgwood. University of Washington, Department of Pediatrics, Seattle; Schneider Children's Hospital, New Hyde Park, NY.

Numerous aberrations in cellular and humoral immunity have been described in JRA including defective antigen (Ag) and mitogen induced lymphocyte proliferation. However, Ag induced antibody (Ab) or mitogen induced lymphokine production have not been systematically studied. To assess the humoral immune response in JRA we injected 8 patients intravenously with bacteriophage ØX 174, a T cell dependent neoantigen, and studied specific Ab responses both *in vivo* and *in vitro*, using a phage neutralization assay. *In vivo* Ab responses to ØX 174 were normal in 3 patients and quantitatively deficient in 5. Seven patients switched from IgM to IgG after secondary immunization, 1 failed to produce IgG antibody. All patients showed deficient *in vitro* Ag induced Ab responses. Spontaneous *in vitro* Ab production was normal in all patients; however, Ag induced Ab responses were deficient in all. Co-culture experiments suggested a B cell defect in 1 patient and a combined B cell/T cell defect in the other 7 patients. *In vitro* interleukin 2 (IL2) production by peripheral blood mononuclear cells using PHA or ConA/PMA stimulation was low in all JRA patients. Anti-T cell antibodies could not be demonstrated in patient sera using indirect immunofluorescence and complement mediated toxicity assays. There was no correlation between degree of humoral immunodeficiency and defective IL2 production, and immunologic abnormalities did not correlate with disease subtype, disease activity or the presence of antinuclear antibodies or rheumatoid factor. The demonstrated immune defects may play a direct role in the pathogenesis of JRA or alternatively represent merely a feature of the disease.

† **990** CIRCUMVENTION OF UNRESPONSIVENESS TO POLYSACCHARIDES IN IMMUNODEFICIENCY DISEASES WITH OLIGOSACCHARIDE-PROTEIN CONJUGATE VACCINES. Richard A. Insel, Porter W. Anderson, Univ Rochester Med Ctr, Dept Peds, Rochester, NY.

Vaccines composed of Haemophilus influenzae b capsular polysaccharide (PRP) oligomers coupled to diphtheria toxoid (DTd), (DTd-ol), have been shown to induce antibodies (Ab) to PRP after repetitive immunization (imm) of infants at ages at which there is no response to PRP vaccine. To further examine the immunogenicity of DTd-ol, a 3-yr-old child with the Wiskott-Aldrich syndrome (WA) and a 7-yr-old child with isolated IgG_2 subclass deficiency (G_2^-) who had both failed to produce Ab following two imms with purified PRP were then imm 3X with DTd-ol. Anti-PRP Ab, μg/ml, increased as follows:

WA: pre-0.07, 1°-0.08, 2°-0.14, 3°-1.1.

G_2^-: pre-0.08, 1°-0.24, 2°-0.91, 3°-3.9.

Both children produced a normal Ab titer to DTd after the first imm. The anti-PRP Ab isotype induced by DTd-ol was almost exclusively the IgG isotype in WA and both IgG and IgM isotypes in G_2^-. Over 90% of the anti-PRP Ab of the IgG isotype was of the IgG_1 subclass in both WA and G_2^-, but IgG_2 subclass Ab did increase after imm in both WA and G_2^- and was not detected preimm. The Ab light chain was predominately kappa type. Analytical isoelectric focusing patterns of Ab showed a restricted number of IgG Ab clonotypes. These results suggest that the poor response to polysaccharides in some immunodeficiency diseases can be bypassed by imm with oligosaccharide-protein conjugate vaccines and offer insight into the response of young infants to DTd-ol.