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EXPANSION OF A MATERNALLY ACQUIRED MONOCLONAL T CELL POPULATION WITH CD3+/CD8+/TCR-1-PHENOTYPE IN A CHILD WITH SCID AND CHRONIC GvHR - POSITIVE TROPISM OF THESE CELLS INTO THE SKIN BUT NOT INTO THE LIVER

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The diagnosis of severe combined immunodeficiency (SCID) was established in a 5 months old boy based on typical clinical and laboratory findings. Analysis of surface markers, however, revealed a remarkable pattern: CD3 86%, CD4 5%, CD8 62%, TCR-1 38%, TCR-2 47%, CD8+/TCR-1+ 21%, CD8+/TCR-2+ 24%. Thus, the CD8+/TCR-1+ population almost absent in normal blood was extremely elevated to 21% of PBMC. To further characterize these cells and the complementary fractions they were double labelled using commercial monoclonal antibodies to CD8 and TCR-1 and sorted by fluorescence activated cell sorting (FACSTAR PLUS). DNA from sorted cells was next analyzed by Southern Blot using a common delta-specific probe. A monoclonal rearrangemnt (Vδ1 to Jδ1) was exclusively found in the abnormally elevated cells but not in any other population analyzed. Using PCR we next generated a clonospecific probe directed against the NDN sequences of the rearranged fragment. Dot blot hybridization with this probe confirmed Southern blot results. The mother's PBMC contained 5% T cells with CD8+/TCR-1+ phenotype which had an identical rearrangement on Southern blot and reacted with the child's clonospecific probe. In addition, the NDN nucleotide sequences were identical. Because of signs of GvHR skin and liver biopsies were taken. Southern blots of DNA extracted from the skin revealed the presence of the rearranged fragment while only a minimal reaction could be observed with liver DNA. This case shows that monoclonal maternal cells can metastasize diaplacentally into the child if they are not destroyed by the child's immune system and that CD8+/TCR-1+ cells may have a role in the skin but not in the liver.

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CALCIUM REGULATED MEMBRANE-ATTACHED HEMOGLOBIN AND MECHANICAL PROPERTIES OF DENSITY FRACTIONATED ERYTHROCYTES HEMBRANES: IMPLICATIONS FOR HEMOLYTIC DISEASES AND RBC AGEING:

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The present study was designed to evaluate the influence of calcium $(Ca)_I$, regulated membrane attached hemoglobin (Hb_m) on the viscoelastic properties of density fractionated human RBC membranes. $(Ca)_I$ of RBC was elevated using ionophore A23187 (10μM); mechanical properties of RBC membranes were determined via micropipette technique. Salient results included: 1) a 300% increase in membrane viscosity of aged cells following ionophore treatment corresponding to elevated Hb_m levels; 2) no change in membrane viscosity and Hb_m levels of young cells after ionophore exposure; 3) no dependence between elastic shear modulus and intracellular calcium concentration. 4) Comparison to osmotically shrunk cells reveals an additional calcium concentration dependent association of hemoglobin to the membrane. This implies membrane mechanical properties are independent from the intracellular viscosity. Our results appear relevant to hemolytic diseases characterized by elevated $(Ca)_I$, (e.g. sickle cell anemia) and to age associated changes in deformability. Supported by DFG Fr 752/1-1 u. 1-2 by NIH grants HL 15162 and HL 15722 and by AHA-GLAA award 537IG.

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ANAPHYLATOXIN C3a-desArg AND COMPLEMENT ACTIVATION PRODUCTS OF THE ALTERNATIVE AND CLASSICAL PATHWAS IN NEONATES

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The levels of complement activation products were measured in EDTA-plasma of term and preterm neonates with gestationalage of 31-42 weeks. The anaphylatoxin C3a-desArg was quantitated with a noval ELISA system using monoclonal antibody reacting selectively with a neoepitope of C3a-desArg. ClrsClInativator and C3b(Bb)P which are generated only upon activation of the classical or alternative pathway, were determined by double sandwich ELISA systems. Plasma concentration of C3a-desArg of normal neonates (180 91ng/ml) did not differ significantly from a control group of adults (171± 76 ng/ml), while C3-levels were significantly decreased compared to adults. C3a-desArg levels in cord blood were markedly diminished (85± 64ng/ml), compared to venous blood, whereas C3 and the activation products ClrsClInactivator and C3(Bb)P showed no differences between cord blood and blood obtained by venipuncture. Neonates with amniotic infection as well as patients with early onset sepsis showed an initial manifold increase of C3a-desArg due to alternative pathway activation. Patients with perinatal asphyxia had only slightly elevated C3a-desArg levels. Thus, C3a-desArg seems to be a highly sensitive and specific indicator for severe neonatal infection.

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HEMATOCRIT AND VISCOSITY REDUCTION OF NEONATAL AND ADULT RED BLOOD CELLS IN NARROW TUBES

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In blood vessels and in artificial tubes with diameters of less than 500 μm, both the blood viscosity and the hematocrit decrease with decreasing tube diameter. The present study was designed to measure the dependence of viscosity reduction on hematocrit reduction of red blood cell (RBC) suspensions of neonates and adults by means of a capillary viscometer. RBC were suspended in buffer solution at hematocrits of 0.40, 0.50 and 0.60l/l. Glass tubes with diameters of 270, 100 and 50 μm were perfused with these suspensions. Tube hematocrit and viscosity decreased significantly at each feed Hct when going from 270 μm tubes to 50 μm tubes. Hematocrit and viscosity reductions were more pronounced for neonatal RBC than for adult cells at each feed hematocrit. At a feed Hct of 0.60 l/l viscosity reduction was 37% for neonates and 29% for adults, whereas at a Hct of 40l/l viscosity reductions were only 30% and 25%, respectively. Hct reductions in 50-μm tubes were 27% for neonates and 20% for adults at a feed Hct of 0.60 l/l and 45% for neonates and 40% for adults at a feed hematocrit of 0.40 l/l. Tube viscosities increased exponentially with increasing tube hematocrit. We conclude that the stronger viscosity reduction of neonatal RBC in 50-μm tubes results from the stronger hematocrit reduction of neonatal cells.

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TSH NEWBORN SCREENING: EUTHYROXINEMIC HYPERTHYROTROPINEMIA = LATENT HYPOTHYROIDISM (LH) ?

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The constellation of TSH elevation with normal T4 is classified as LH, by some authors also in newborns. This suggests, that with considerable probability manifest hypothyroidism (MH) will develop.

In a prospective study on 91509 5-day-old-newborns we determined TSH by the RIA of Becton & Dickinson.

570/91509 showed TSH >20 mU/l. In follow-up 40/570 showed persisting TSH elevation, and in addition T4 <6 ug/dl. Thus these 40 had MH. One of the 570 could not be followed up. Of the other 529: 507 further on had normal TSH and T4, in 22/529 TSH persisted elevated, T4 normal. 17/22 had continuously normalizing TSH (T4 always normal). Only in 5/22 TSH persisted elevated, T4 decreasing towards the lower limits of normal. In all five thyroid scan showed lingual ectopia, thus MH (treatment was started before T4 fell below the lower limits of normal).

Thus out of 529 neonates with euthyroxinemic hyperthyrotropinemia TSH elevation was only transient in more than 99 % (524/529), no MH developed. From these results we conclude that it is not adequate to classify every newborn with euthyroxinemic hyperthyrotropinemia as LH.

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LIPID LEVELS IN PLASMA AND MONOCYTES (MO) IN CHILDREN WITH END-STAGE RENAL DISEASE (ESRD).

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Patients (pts) with ESRD frequently have atherogenic lipoprotein profiles. In early atherosclerotic lesions, monocyte-derived macrophages develop into foam cells through accumulation of cholesterol (Chol). We measured lipoprotein profiles in plasma and lipids in MO of 6 pts (age 14.4±3.2 y) treated by peritoneal dialysis (PD), 10 pts (age 15.4±4.3 y) treated by hemodialysis (HD) and 7 controls (Ctr; age 22.8±10.4 y). PD and HD pts had elevated levels of plasma triglycerides (TG), plasma Chol (PD only), and Chol in the VLDL and LDL fraction (PD only), whereas HDL-Chol levels were low. Apolipoprotein (APO) B, but not APO AI, plasma levels were significantly elevated only in PD pts. In MO, we measured free Chol (FC), Chol esters (CE), and TG (MO/TG). Results (ug/mg cell protein; x±SD):

	PD	HD	Ctr
FC	50.1(±12.2)	30.8(±11.2)	53.0(±11.8)
CE	2.5(±1.4)	1.5(±1.0)	1.4(±0.7)
TG/MO	2.5(±1.2)	3.3(±0.9)	3.0(±2.3)

We conclude that increased lipoprotein lipid levels are not associated with lipid accumulation in MO. Only pts treated with HD have a significant decrease of FC in MO.