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DISACCHARIDASE ACTIVITIES IN INFANTS: NORMAL VALUES AND COMPARISON BASED ON SYMPTOMS AND HISTOLOGICAL CHANGES.

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Background: There is an uncertainty and paucity of data regarding normal levels of disaccharidase activities (DA) in infants. **Aim:** To establish normal values for DA in infants. To determine the relationship between symptoms, intestinal mucosal histology and DA. **Methods:** Histology and DA of intestinal mucosal specimens from 131 infants (75 males; mean age 180 d; age range, 20-364 d) obtained endoscopically over an 8 year period were reviewed. Patients were divided into 2 groups based on absence (Group 1; n=63) or presence (Group 2; n=68) of failure to thrive (FTT) and/or diarrhea. These groups were further subdivided into 3 subgroups based on histological findings: (normal: A, mild abnormalities: B, and moderate/severe changes: C). **Results:** DA from group 1A represent normal values as these infants were free of FTT/diarrhea, and had normal intestinal mucosal histology (Table). Differences in DA were not dependent on symptoms, in absence of histological abnormalities (groups 1A and 2A), but rather on presence of histological changes even in the absence of symptoms (groups 1A vs 1B). Also, differences were found when patients with FTT and/or diarrhea with abnormal histology were compared to patients with no FTT and/or diarrhea with a normal brush border (groups 2B and 2C vs 1A). **Conclusions:** We describe normal levels of DA in infants. These may serve as reference values for clinical practice and laboratory analyses. Additionally, DA were related to mucosal histology and not certain symptoms.

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GROWTH OF INFANTS WITH BRONCHOPULMONARY DSPLASIA AFTER DISCHARGE.

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Purpose of Study: The objective of our study is to compare the pre- and post-discharge growth of infants with severe BPD enrolled in the LUMC Neonatal Home Care program to the growth after discharge of other infants with mild BPD seen at the neonatal follow-up clinic.

Design and Methods: Adequate growth velocity of premature infants is 26 to 40 grams per day at one month corrected gestational age (GA). In this prospective study, 70 infants admitted to LUMC NICU from 9/1/04 until 10/31/05 with a birth weight ≤ 1500 grams were enrolled. Infants with an O2 requirement for ≥ 28 days were selected. LUMC Home Care Program follows infants discharged home requiring O2, NG feedings, monitors or special needs. Infants requiring O2 at home were the severe BPD group, and infants without home care and on room air were the mild BPD group. While in the NICU, length, weight and head circumference were recorded weekly in nutrition rounds. After discharge, the severe BPD group had their weight and length recorded at each home visit up to 6 months corrected GA, according to the severity of their disease. The mild BPD group had data collected at the NICU Follow-Up Clinic. Feedings and average daily calories were recorded.

Results: 70 patients completed the study. 39 were in the severe BPD group, and 31 were in the mild group. The data was analyzed with the student t-test.

Table 1	Severe BPD (39)	Mild BPD (31)	P-value
Pre-Discharge Weight Gain (gm/kg/day)	21.25	23.83	0.015
Pre-Discharge Length Gain (cm/week)	0.93	0.91	0.857
Post-Discharge Weight Gain (gm/kg/day)	36.63	36.13	0.913
Post-Discharge Length Gain (cm/week)	1.18	1.31	0.365

Conclusion: While in the NICU, the infants in the severe group had decreased weight gain compared to those with mild BPD. After discharge, the infants with severe BPD gained weight as rapidly as those in the mild group. The change in length before and after discharge was not statistically significant. Our study shows that with proper follow-up, infants with severe BPD can grow as well as infants in the same birth weight group but with milder disease.

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INCREASED TH2 ACTIVITY *IN VIVO* PROMOTES THE DEVELOPMENT OF ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that predisposes towards the development of subsequent atopic phenotypes. The pathogenesis of AD is still poorly understood and likely is due to defects in skin and immune function. In the present study we focus on how Th2 development promotes atopic dermatitis.

It is well documented that Stat6 plays a pivotal role in the activation of Th2 cytokines including IL-4 and IL-13. Recent studies from our laboratory have shown that transgenic mice expressing active Stat6 (Stat6VT) in T cells have increased Th2 differentiation *in vivo* and *in vitro*. All Stat6VT transgenic mice developed severe blephritis (inflammation around the eye) characteristic of IL-4 over-expressing mice. Furthermore, 30-50% of the Stat6VT transgenic mice developed the characteristic symptoms of atopic dermatitis involving erythema and inflammation of different organs such as ears, nose, face, neck and tail. Histological analyses of tissue samples from mouse ears of Stat6VT transgenic mice revealed dermal infiltration of lymphocytes and eosinophils, whereas neutrophils appeared prominent in more severe lesions. This may reflect a skewing of Th2 to Th1 inflammation in more chronic lesions.

To define the importance of IL-4 in these phenotypes, we mated Stat6VT transgenic and IL-4-deficient mice (Stat6VT/IL-$4^{-/-}$). T cells from Stat6VT/IL-$4^{-/-}$ mice were still capable of becoming Th2-like cells, secreting IL-5 and IL-13. However, blephritis in Stat6VT/IL-$4^{-/-}$ mice occurred less frequently and with only mild symptoms. Stat6VT/IL-$4^{-/-}$ mice did not develop atopic dermatitis. These results suggest that, in contrast to the role of IL-13 in asthma, IL-4 is an effector cytokine in these allergic conditions.

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CHEMOPROTECTION OF LONG-TERM REPOPULATING HEMATOPOIETIC STEM CELLS FROM ALKYLATOR THERAPY: *IN VIVO* COMPARISON OF GENE-TRANSFER VECTORS THAT EXPRESS A DNA REPAIR PROTEIN.

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Dose-intensification of alkylator-based chemotherapy in cancer patients can result in life-threatening cytopenia. The DNA repair protein, O^6-methylguanine DNA methyltransferase (MGMT), repairs DNA damage mediated by alkylating agents in hematopoietic stem cells (HSC). Two viral vector systems for the transfer and expression of the MGMT DNA repair protein in HSC were compared in vivo. A foamy virus vector that can transduce noncycling HSC was compared to an oncoretroviral vector that can transduce HSC induced into cell cycle by cytokine stimulation. The virus vectors express a mutant form of MGMT called $MGMT^{P140K}$ that protects HSC from high-dose alkylator therapy. Lineage-depleted bone marrow (BM) from C57BL/6 mice was transduced for 10-16 hours with the foamy virus vector or following a 2-day pre-stimulation with the oncoretrovirus vector. The bulk transduction efficiency using the foamy virus vector ranged from 12-25% and the progenitor transduction efficiency was 55-57%. Transductions with the oncoretrovirus vectors resulted in similar bulk and progenitor transduction efficiencies (55-60%). Data are from three primary and two secondary transplant experiments. Transplantation of oncoretroviral vector-transduced cells resulted in 90-95% of the cells expressing $MGMT^{P140K}$ in the PB and BM in primary and secondary recipient mice following 2-3 cycles of alkylator therapy. In mice transplanted with foamy virus-transduced cells, $MGMT^{P140K}$ was elevated at 6 months post-transplantation but protection was not as robust as in mice transplanted with the oncoretrovirus-transduced cells. Approximately 50% of the progenitor colonies contained the foamy provirus following in vivo selection. In secondary reconstitution experiments, $MGMT^{P140K}$ expression remained elevated in the BM of mice transplanted with oncoretrovirus- or foamy virus vector-transduced cells. These data demonstrate that although foamy virus transduction is not quite as efficient as the oncoretrovirus transduction strategy, a simple overnight protocol using a foamy virus vector can be used to transduce minimally stimulated HSC. Studies are in progress to further dissect the requirements for efficient transduction of HSC with foamy virus vectors.

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LIMITED ABILITY OF LONG-TERM CULTURED MURINE MARROW-DERIVED MESENCHYMAL CELLS TO SUPPORT HEMATOPOIESIS

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We studied the growth, differentiation and ability to support hematopoiesis of long-term cultured murine marrow-derived mesenchymal cells (MC) cultured in serum-containing medium but without additional cytokines. Unfractionated marrow from C57Bl6/J (Bl/6; CD45.2+) mice was plated in DMEM with 10% bovine serum; adherent cells were passaged when >80% confluent. During early (<6 weeks) culture, adherent cells were ~50% CD45+ and showed variable morphology. After 6-8 weeks of slow growth (<1 passage/week) a population of fibroblast-like, rapidly dividing (~24h doubling time) cells emerged, which we have continuously cultured for at least 8 months. These cells express a surface phenotype (≤2% CD45+; ≥90% CD29, 90, 105, 106+) consistent with MC, and are capable of osteogenic and adipogenic differentiation in vitro. Analysis of five clones derived from one long-term MC culture showed similar growth characteristics as the parental culture, but the clones displayed variability in surface phenotype and the ability to undergo adipogenic differentiation. Furthermore, 2/5 clones were unable to undergo osteogenic differentiation, indicating that long-term MC cultures are comprised of heterogeneous subpopulations of cells. Transplantation of 10^6 long-term cultured MC with $5x10^5$ unfractionated B6.SJL-PtrcaPep3b/BoyJ (BoyJ; CD45.1+) marrow cells into ablated BoyJ hosts revealed <2% MC-derived CD45.2+ blood cells four months post-transplant, demonstrating that long-term cultured MC lack inherent hematopoietic potential. Marrow cells co-cultured on long-term cultured MC feeder layers in alpha-MEM with 20% serum produced ~5-fold more hematopoietic colonies in CFU-C assays than marrow cultured without MC feeders. Marrow cells co-cultured on MC displayed >5-fold lower repopulating ability, however, compared to marrow cells cultured without MC feeders, four months post-transplant in two independent competitive repopulation assays. These data suggest that long-term cultured MC, though morphologically and phenotypically homogeneous and lacking hematopoietic potential, may not support ex vivo culture/expansion of primitive hematopoietic cells, at least under these stringent (i.e., without additional cytokines) conditions. Assays comparing the hematopoiesis-supporting capacity of long-term cultured MC to classic Dexter-type stroma, and studies to determine optimal co-culture conditions are ongoing.

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GADD45γ – ESTABLISHING A ROLE FOR THE CELL CYCLE IN SOMITOGENESIS.

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In vertebrates the repeated metameric pattern of the axial skeleton is evident early, demonstrated by the serially repeated segments known as somites. Somites are blocks of mesodermal tissue that separate from the undifferentiated presomitic mesoderm (PSM) in a process that is highly regulated. The somites will go on to form several critical structures in the embryo, including the vertebrae and musculature of the axial skeleton. Multiple pathways controlling somite segmentation have been identified, including pathways involving FGF8, retinoic acid, NOTCH and WNT signaling. Previous investigators have also suggested a role for the cell cycle in segmentation, but no molecular evidence for this has existed to date.

We have identified a cell cycle inhibitor – Gadd45γ – that appears to be involved in somitogenesis. Gadd45γ is a growth arrest gene that controls the G2/M cell cycle checkpoint. Gadd45γ is expressed in the somites S0 and S-1 throughout somitogenesis, in the region where cells are moving from the undifferentiated presomitic mesoderm to the differentiated somite. Embryo culture experiments and analysis of mouse mutant lines demonstrates that Gadd45γ is regulated by the major pathways involved in somite formation, including FGF8, retinoic acid and Notch. Overexpression of Gadd45γ in mouse embryos disrupts normal segmentation and results in decreased expression of Mesp2, a key player for establishing normal somite boundaries. Further work is underway to determine if loss of Gadd45γ function results in segmentation defects. Gadd45γ appears to play a role in segmentation and establishes a link between the cell cycle and the segmentation clock.

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