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FLUORESCENTLY TAGGED PROTEINS TO MONITOR NADPH OXIDASE ASSEMBLY DURING PHAGOCYTOSIS

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The ability to examine the spatial and temporal localization of proteins in living cells through the use of fluorescent tags is a powerful tool for investigating the organization and dynamics of many cellular processes. We are applying this technology to studies on the NADPH oxidase expressed in neutrophils and monocyte/macrophages. This enzyme catalyzes the first step leading to the generation of potent microbicidal oxidants, which are critical for the killing of many bacterial and fungal pathogens. The importance of the NADPH oxidase to host defense is attested to by the impact of genetic defects in this enzyme, which result in Chronic Granulomatous Disease. In order to better understand the assembly and molecular regulation of this multi-subunit enzyme during phagocytosis, we are analyzing and testing fluorescently tagged derivatives of the NADPH oxidase subunits. The assembly of cytosolic factors $p47^{phox}$ and $p67^{phox}$ with flavocytochrome b_{558} ($gp91^{phox}$ and $p22^{phox}$) at the membrane is a crucial step in enzyme activation. The $p47^{phox}$ and $p67^{phox}$ subunits are linked via a high-affinity, tail-to-tail interaction involving a proline-rich region (PRR) in an SH3 domain, respectively, in their C-termini. This interaction is critical for $p47^{phox}$ dependent $p67^{phox}$ membrane translocation. To investigate molecular rearrangements of $p47^{phox}$ and $p67^{phox}$ during oxidase activation, a fragment derived from the $p47^{phox}$ PRR and tagged with YFP (p47/YFP) was generated and shown to bind to recombinant $p67^{phox}$ in vitro. When expressed in PLB-985 granulocytes, this fragment was recruited to phagosomes. This suggests that the C-terminal SH3 domain of $p67^{phox}$ becomes accessible following oxidase assembly. Additional studies are examining the role of $p40^{phox}$, an additional regulatory subunit of the NADPH oxidase that has a phosphoinositide binding site critical for oxidase activity on phagosomes (Suh, et al, *J Exp Med*, In Press). Whether $p40^{phox}$ also promotes recruitment of $p67^{phox}$ to the membrane or acts as an allosteric regulator is being examined in similar studies using fluorescently tagged derivatives while imaging living granulocytes undergoing phagocytosis.

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TRENDS IN SINUS OF VALSALVA DILATION AFTER THE ROSS PROCEDURE.

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Purpose: Since its original description in 1967 by Dr. Ross, the use of a pulmonary valve autograft for aortic valve replacement is becoming more prevalent. We analyzed pre and post operative data of pts who underwent the Ross procedure at Riley Children's Hospital in Indianapolis Indiana in order to follow trends of survival, reoperation, outflow valve integrity and aortic root dilation. This is a sub-analysis of nine patients who had multiple serial echocardiograms available for review. **Methods:** Clinical and echocardiographic data were reviewed on 81 consecutive children (n=66) and adults (n=14) that had a Ross procedure between 1993 and 2005. Median age at the time of the procedure was 13 years (range 9d to 36 years). A sub-analysis of nine patients who had serial echocardiograms available on our digital data base was performed using 2D measurements of the aortic annulus, sinus of valsalva and sino-tubular junction. The median age of these patients was 14 years (range 18mo-18 years). Follow up from surgery to the last echocardiogram was a median time of 4 years (range 650 days to 2302 days). **Results:** Almost 47% of patients developed dilation of the sinus of valsalva beyond the 95% of normal by late follow up ($p<0.0001$ late vs early dilation). These nine patients all showed dilation of the sinus of valsalva greater than the 95% of maximum (range 1.01 to 1.52 times the upper limits of normal). However, rate of growth of the sinus of valsalva did not statistically differ from the rate of growth in the normal population ($p>0.05$).**Conclusions:** The Ross procedure is a good alternative to artificial valve replacement for aortic diseases, however dilation of the sinus of valsalva is common, but trends don't appear to exceed the rate of normal growth.

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RENAL TRANSPLANT SURVEILLANCE BIOPSY RESULTS IN CHILDREN RECEIVING STEROID FREE IMMUNOSUPPRESSION

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Introduction: Interstitial fibrosis (IF) is frequently present in renal biopsy specimens obtained from transplant recipients who receive tacrolimus (TAC) and prednisone as maintenance immunosuppression, especially in those in whom the associated trough TAC levels are elevated. In contrast, little information exists on the kidney histology of patients on a steroid free (SF) immunosuppression regimen. The role that the exposure to TAC [trough levels or area under the curve (AUC)] has on biopsy findings remains poorly defined. **Aims:** To report the 6 month renal transplant surveillance biopsy results of patients receiving SF immunosuppression. The relationship between TAC usage and the biopsy findings was also investigated. **Methods:** Medical records from 14 pediatric renal transplant recipients who received a maintenance immunosuppression regimen consisting of TAC & mycophenolate mofetil were reviewed for age at time of transplant, gender, ethnicity, donor type, cold ischemia time, TAC dose and trough level and estimated GFR at time of biopsy. TAC AUC was assessed in 7 patients. Data is presented as mean ($\pm$SD). Results: Of the 14 patients (mean age: 11.8$\pm$5.23 yrs), 10/14 (71%) were caucasian, 9/14 (64%) were male, and 10/14 (71%) received a living related donor transplant, with a cold ischemia time of 7.66$\pm$8 hrs. Surveillance biopsies were performed at a mean time of 200.92$\pm$33.3 days post transplantation. Serum creatinine at the time of biopsy was 0.94$\pm$0.46 mg/dl with a Schwartz estimated GFR of 86.88$\pm$34.29 ml/min/1.73 m^2. TAC dose at time of biopsy was 0.17$\pm$0.25 mg/kg/day and the associated trough level was 6.36$\pm$1.52 ng/ml. In the 7 patients who had done TAC pharmacokinetics, the mean C_0 was 6.75$\pm$1.28 ng/ml and AUC 110.8$\pm$32.37 [h x (ng/ml)]. Based on the Banff '97 working classification of renal allograft pathology, interstitial fibrosis (IF) involved a mean 17.3$\pm$7.66% of the biopsy specimen and was present in 10/14 (71%) biopsies. There was no correlation found between trough TAC level and % IF. A mean of 18.75$\pm$3.77 % IF was found in those who had TAC pharmacokinetics performed. **Conclusion:** IF is frequently found in "well-functioning" renal transplants as early as 6 months post transplant in association with a SF, TAC based immunosuppression regimen in children. The potential correlation between transplant histology, trough TAC levels and TAC pharmacokinetics requires further study in a larger number of patients.

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INVASIVE BACTERIAL INFECTIONS CAUSED BY EXTENDED-SPECTRUM B-LACTAMASE (ESBL) PRODUCING MICROORGANISMS IN NEONATAL INTENSIVE CARE UNIT (NICU) SETTING.

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Purpose: Recently, we have noticed an increased incidence of infections caused by antibiotic-resistant pathogens including ESBL-producing microorganisms in our NICU patients. The purpose of this study was to investigate the incidence, predisposing conditions and treatment outcome of ESBL-producing *Klebsiella* species and *E. coli* invasive infections in our neonatal intensive care unit population.

Methods: Review of medical and microbiological records of neonates with invasive infections caused by *Klebsiella* and *E. coli* who were treated at Children's Hospital of Michigan NICU during a 2-year period.

Results: During the 2-year study period (January 2003-December 2004), 88 patients with positive cultures for *Klebsiella* or *E. coli* were identified. Of the 88 positive cultures, 52 were determined to be true infections and 36 were considered to represent colonization. The 52 infected patients had a median age of 30 days (3-300 days) and 27 (52%) were male. Infecting organisms were recovered from blood in 16 (30.8%), CSF 1 (1.9%), peritoneal fluid 2 (3.8%), lung tissue 2 (3.8%), bronchoalveolar lavage 1 (1.9%), urine 20 (38.5%), sputum 7 (13.5%) and open wound swabs 3 (5.8%). *E. coli* was isolated from 24 patients (46.2%), *K. pnemoniae* 19 (36.5%), *K. oxytoca* 5 (9.6%) and *E. coli* plus *K. pnemonia*e from 4 (7.7%). Six of the bacteremic patients had at least one other positive culture. Among the bacterial isolates, 1/24 (4.2%) *E. coli*, 10/19 (52.6%) *K. pneumoniae* and 2/5 (40%) *K. oxytoca* were ESBL enzyme producers. Logistic regression analysis indicated that only treatment with 3rd or 4th generation cephalosporin during the 3 months prior to onset of infection was associated with an increased risk of acquiring ESBL-producing *Klebsiella* or *E. coli* infections ($P=.002$; OR 14.0; CI_{95}, 2.6 to 73.6). All except one ESBL-producing isolate were susceptible to the two carbapenems, imipenem and meropenem (MIC$\leq 4\mu$g/ml). Among 13 patients with infections caused by ESBL-producing microorganisms, 5 were treated with a carbapenem and 4 improved; 8 were not treated with a carbapenem and 4, who were bacteremic, died.

Conclusions: Half of the *Klebsiella* isolates that caused infections in our NICU patients were ESBL-producing strains. Treatment with 3rd or 4th generation cephalosporins within the previous 3 months was associated with an increased risk of acquiring ESBL-producing *Klebsiella* or *E. coli* infections. Treatment of ESBL-producing *Klebsiella* or *E. coli* infections with a carbapenem was associated with a favorable outcome in our patients.

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SACROSIDASE IN THE TREATMENT OF CHRONIC NONSPECIFIC DIARRHEA IN CHILDREN.

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Context: Chronic nonspecific diarrhea in children, also known as toddler's diarrhea, is a frequently encountered disorder in clinical practice. It tends to cause significant parental anxiety and frustration leading to frequent health care visits. **Objective:** We investigated the usefulness of sacrosidase (Sucraid®) in children with toddler's diarrhea. The primary outcome was the proportion of patients who responded to sacrosidase supplementation. Secondary outcome measures included safety and potential adverse effects. **Population:** Children 1 to 6 years of age with a current history of toddler's diarrhea ($\geq$ 3 loose stools per day for $\geq$ 3 months) were enrolled in a prospective trial from our outpatient specialty clinic. Exclusion criteria included a history consistent with infection, inflammation, malnutrition or the use of motility altering agents. Patients were also excluded if they had abnormal screening tests including the presence of anemia, elevated inflammatory markers, positive celiac disease antibodies or infectious stool studies. **Design:** Baseline defecation patterns (stool frequency and consistency) were obtained using daily diaries filled by caregivers over 1 week prior to any intervention. Patients with baseline defecation patterns that satisfied the definition of toddler's diarrhea ($\geq$ 3 stools daily) were placed on sacrosidase supplementation with all snacks and meals. Caregivers filled similar daily diaries for 2 weeks. We defined clinical response as a decrease in daily stool frequency by 50% and/or a decrease of $\geq$ 2 points on the stool consistency scale while on sacrosidase supplementation. **Results:** Between September 2005 and July 2006, 37 children presented for evaluation of diarrhea, of which 20 were eligible for the study. Of those eligible, 12 were enrolled (average age 31.6 months, range 41 to 23 months). One subject was excluded from analysis for noncompliance and three subjects were not kept in the study due to normal defecation patterns in the baseline period. Four of the remaining eight patients (50%) who finished the study were clinical responders. In the responder group, the average daily stool frequency decreased from 5.2 to 1.9 and the stool consistency scale decreased from a mean of 4.5 to 2.5 points. In the non-responder group, the average daily stool frequency and stool consistency remained unchanged. There were no complications reported. **Conclusions:** Sacrosidase supplementation demonstrated a potential benefit in children with toddler's diarrhea by decreasing defecation frequency and improving stool consistency. This may be due to excessive sucrose in children's diet or to unrecognized partial sucrase deficiency. A larger scale study with longer patient follow up is needed to further assess the benefit of such an intervention.

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BONE DENSITY IN CHILDREN WITH HEMANGIOMAS TREATED WITH SYSTEMIC GLUCOCORTICOIDS.

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Purpose: To evaluate the long-term effects of systemic glucocorticoid therapy on bone mineral status in children previously treated for complicated infantile hemangiomas. **Methods:** Bone density was evaluated by tibial ultrasound (Omnisense 7000P) and DEXA bone scan (Lunar Prodigy 10667) in children with hemangiomas previously treated with glucocorticoids (off treatment $\geq$ 1 year) and healthy controls. In addition, Tanner stage, bone age, and body mass index (BMI) were determined for all patients. **Results:** The control group consisted of 24 patients with a mean age of 52 months (range 16-104). The treated group consisted of 30 patients with a mean age of 44 months (range 20-75). Mean duration of glucocorticoid treatment was 8.6 months (range 4-15.5) and mean maximum dose of prednisolone was 2.2 mg/kg/day (range 1-4.3). All patients were Tanner stage I and there were no significant differences in bone age or BMI between the treated and control groups. Mean spinal (L2-L4) bone mineral density (BMD) did not differ between the treated group (0.6 g/m2, S.D.=0.07) and the control group (0.58 g/m2, S.D.=0.11) (95% C.I. for mean difference of -0.07 to 0.03). There were also no significant differences between treated and control groups for mean total body BMD or tibial ultrasound z-scores. **Conclusion:** Preliminary data analysis reveals no significant differences in bone density between children who received systemic glucocorticoids and children who did not.