**TUBULIN (Tub) POLYMERIZATION MODULATES INTERLEUKIN-2 (IL-2) RECEPTOR (R) SIGNALING IN T-CELLS**

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Previous work shows that cytoskeletal proteins like Tub are involved in signaling via the T-cell antigen R and co-Rs like CD4, but their role in cytokine R signaling has not been assessed. We thus studied IL-2 R signal transduction, a target of new immunosuppressants, in the context of taxol(Tax)-augmented or colchicine(Col)-attenuated cytoskeletal Tub polymerization. Human T-cells were isolated by density-gradient centrifugation and sheep erythrocyte rosetting, stimulated with low-dose PHA, acid-stripped and rested before a 3hr-incubation in Tax or Col (0.5 to 50$\mu$M in DMSO), followed by IL-2 stimulation (100U/ml, 20 min). Immunoblots of cell lysates demonstrated dose-dependent inhibition of IL-2-induced tyrosine phosphorylation of unidentified proteins after Col pretreatment but not DMSO alone, while Tax preincubation augmented it. Gel shift assays with whole cell extracts of the cells showed augmented formation of IL-2-induced DNA-binding protein by Tax, while Col again decreased it. As IL-2 signaling regulates IL-2 R $\alpha$-chain expression, we examined by FACS if Tax or Col applied as above modulate IL-2 R $\alpha$-chain expression but found no effects. Furthermore, neither agent affected cell viability by Trypan Blue exclusion. These studies document a novel role for cytoskeletal Tub in T-cell signaling, as Tub polymerization is shown to influence IL-2 R signaling without affecting IL-2 R $\alpha$-chain expression. Studies to identify elements of IL-2 R signal transduction cascades affected by Tub polymerization are ongoing to better understand immunomodulatory strategies with Tax, Col or new immunosuppressants. (Univ. of Kentucky Med. Center Research Fund grant support)

**Factors associated with Pre-Event Perceived Coronary Heart Disease Risk in Women**

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Recent surveys indicate that women fail to perceive that they are at risk of developing or dying from coronary heart disease (CHD). We surveyed a population of 59 women(60 $\pm$10yrs)who were within 5.3$\pm$10 months of an index cardiac event.**Methods:**A self reported questionnaire was administered with respect to their pre-event perception of CHD risk using a 1-10 rating scale, and CHD risk factors(family history,smoking,elevated cholesterol,hypertension and sedentary activity). They were also evaluated with regard to their belief to the most common cause of death in women.**Results:**Sixty-One percent of the women had a lower pre-event perception(LPEP) of CHD risk (Rank 1-5) compared to 39% of the women who had a higher pre-event perception(HPEP)(Rank 6-10). The women in the HPEP group were more likely to perceive themselves as sedentary(78%vs50%)and obese(76%vs50%)as compared to women in the LPEP group($p<.05$). There were no significant differences with regard to hypertension(61%vs61%),family history(74%vs50%)elevated cholesterol(36%vs45%) or unknown cholesterol(35%vs15%) as compared to women in the LPEP group($p<.05$).**Conclusions:**1)Women within the LPEP group differ from the HPEP group with significantly lower self reported obesity and sedentary behavior. 2)Women who perceive breast cancer to be the most common cause of death in women are more likely to have a lower pre-event perception of their own CHD risk.

TREATMENT WITH PEPTIDE HORMONES INCREASES MACROPHAGE Fcγ RECEPTORS EXPRESSION. F. Gomez and P. Ruiz. Department of Medicine. Hospital Universitario de Puerto Real/S.A.S. University of Cadiz. School of Medicine. Spain.

Macrophage Fcγ receptors (Mø-FcγR) are important in the pathophysiology of immune cytopenias and, in host defense against infection. Thus, the regulation of Mø-FcγR expression is a therapeutic target in immune mediated disorders.

We have utilized an experimental model in the guinea pig to assess the effect of prolactin (PRL), thyroid stimulating hormone (TSH), insulin (I), corticotrophin (ACTH), thyroid hormone (T4) and growth hormone (hGH) on: (1) the splenic macrophage FcγR clearance of EA ($^{51}$Cr labeled guinea pig RBCs sensitized with defined amounts of IgG rabbit anti-guinea pig RBC antibody, (2) the surface expression of both FcγRs expressed by guinea pig macrophages assessed by FACS analysis with specific monoclonal antibodies against those FcγRs, FcγR1,2 and FcγR2, and (3) the function of Mø-FcγRs, FcγR1,2 and FcγR2, studied by the *in vitro* binding of rabbit RBCs sensitized with IgG1 (EA1) or IgG2 (EA2) guinea pig anti-rabbit RBC antibodies. FACS analysis and binding of EA1 or EA2 were also performed after culture of isolated guinea pig splenic macrophages with the above hormones.

PRL, TSH, I, T4 and hGh enhanced the clearance of EA, the binding of EA1 or EA2, and the surface expression of both FcγR1,2 and FcγR2. Whereas, ACTH had reciprocal effects on Mø-FcγR expression. Isolated splenic macrophages cultured for 72 hours with PRL, TSH, Y, ACTH, T4 or hGH showed an enhanced expression of both FcγR classes, FcγR1,2 and FcγR2 and, an increased binding of EA1 or EA2. FcγR ,2 was more sensitive to regulation than FcγR2.

Treatment with peptides hormones, PRL, TSH, I, T4 and hGH, increase the expression of macrophage FcγRs. ACTH *in vitro* increases macrophage FcγR expression, while *in vivo* treatment with ACTH inhibits macrophage FcγR expression.

PEPTIDE HORMONES REGULATE HUMAN MACROPHAGE Fcγ RECEPTORS EXPRESSION IN VITRO. F. Gomez and P. Ruiz. Department of Medicine. Hospital Universitario de Puerto Real/S.A.S. University of Cadiz. School of Medicine. Spain.

The immune system is under neuroendocrine influences. Macrophage Fcγ receptors (FcγR) are important for host defense and the pathophysiology of immune cytopenias and immune complex diseases. The modulation of macrophage Fcγ receptors expression is a therapeutic target for immune mediated disorders. We have studied the effect of peptide hormones: prolactin (PRL), thyroid stimulating hormone (TSH), insulin (I), corticotrophin (ACTH), thyroid hormone (T4) and growth hormone (hGH) on the *in vitro* expression of macrophage Fcγ receptors.

Adherence purified peripheral blood monocytes were cultured for 72 hours in the presence or absence of PRL, TSH, I, ACTH, T4 or hGH. Fcγ receptors expression was assessed by FACS analysis using specific monoclonal antibodies against the three types of FcγR expressed by cultured human monocytes: anti-FcγRI (MoAb 32.2), anti-FcγRII (MoAb IV.3) and anti-FcγRIII (MoAb 3G.8). Monocytes cultured with PRL, TSH, I, ACTH, T4 and hGH showed enhanced expression of all FcγR classes: FcγRI, FcγRII and FcγRIII. The expression of FcγRIII was more increased than the expression of FcγRI or FcγRII. The binding of IgG sensitized cells by cultured monocytes was also increased by PRL, TSH, I, ACTH, T4 and hGH. PRL, TSH and hGH were more potent upregulators of macrophage FcγR expression than I, ACTH and T4.

Human peripheral blood monocytes cultured for 3 days with prolactin, thyroid stimulating hormone, insulin, corticotrofin, thyroid hormone (T4) and growth hormone show enhanced expression of the three Fcγ receptors classes: FcγRI, FcγRII and FcγRIII. Additional work should be done to assess the *in vivo* effects of the above hormones on the expression of FcγR by macrophages.

A NOVEL FLOW CYTOMETRIC METHOD FOR THE QUALITATIVE ANALYSIS OF P53 PROTEIN ELEVATION IN GLIOBLASTOMA MULTIFORME. ON Gottfried, DL Way and AJ Hamilton (SPON: MH Witte). Dept. of Surgery, University of Arizona College of Medicine, Tucson, AZ 85724.

We herein demonstrate a new flow cytometric technique, utilizing frozen, non-fixed glioblastoma multiforme tumor (GBM) tissues, for the assessment of intra-nuclear p53 protein levels. Eleven surgically resected neural tumors, previously diagnosed by histochemistry as GBMs, were selected from snap-frozen, archival material. Thawed samples were mechanically disaggregated and the released nuclei filtered removing contaminating debris. Nuclei were labeled with fluorescent monoclonal antibodies specific for human p53 protein. Nuclear fluorescence was quantitated by flow cytometry (FCM) and p53 protein content determined as percent of control. Eleven of Eleven GBMs were successfully analyzed with the resulting histograms routinely containing 10,000 nuclei. Ten of the eleven GBMs (91%) had elevated levels of p53 protein. Previously reported immunohistochemical data from paraffin embedded, fixed sections suggest p53 elevation in two-thirds of all GBMs. Our increase in the number of GBMs found to have elevated p53 levels may represent the objectivity and accuracy of FCM in addition to the p53 preservation effect inherent in frozen tissues. Additionally, our use of frozen tumors and biopsy-sized samples offers rapid, convenient, and reliable determinations of p53 protein status. This procedure allows for efficient, precise, and controlled p53 analysis on frozen biopsy-sized samples quickly identifying potential candidates for gene therapy, radiation therapy, and providing information important for appropriate chemotherapeutic delivery in the treatment of this devastating disease.

PTHrP INDUCED HYPERCALCEMIA IN SQUAMOUS CELL CARCINOMA OF HEAD AND NECK AFTER RADIATION. A. Gover, P. Dileep Kumar and Keyvan Ravakhah (SPON: Burton C. West). Dept of Medicine, Huron Hospital, Cleveland, OH 44112.

Squamous cell carcinoma of the head and neck rarely causes humoral hypercalcemia of malignancy. We report a case of squamous cell carcinoma of the oral cavity which produced PTHrP causing hypercalcemia only after radiotherapy and chemotherapy.

65 y/o African American woman presented with pain and swelling of the lower jaw. Exam showed a fungating mass of the floor of the mouth with enlarged submandibular and cervical lymph nodes. Biopsy showed poorly differentiated squamous cell carcinoma. Serum calcium was 8.3 mg/dL. She underwent radiation therapy, chemotherapy & later tumor resection. Eight months after the first presentation she became lethargic, calcium level rose to 15.6 mg/dL (8.5-10.3), parathyroid hormone 37 pg/mL (11-54) and PTHrP 16.6 pmol/L (0-1.5). She was treated with saline & pamidronate. She expired of asphyxia from upper airway obstruction.

Our case is unique since malignant cells initially did not produce PTHrP. This is suggested by the absence of hypercalcemia despite a large tumor volume. Later, clonal rearrangement or amplification of the PTHrP gene of the tumor cells might have lead to the production of PTHrP. Whether chemo- or radiation therapy contributed to this event or it occurred *de novo* is unclear.