343 The Influence of Pioglitazone on NFκB Expression in CD40+ Lymphocytes is Polymorphism-Dependent

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RATIONALE: CD40, a co-stimulatory protein found on antigen presenting cells, binds CD154 (CD40L) on T helper lymphocytes initiating intracellular signal cascades involving NFκB. PPAR-γ is a family of nuclear receptors with anti-inflammatory effects which antagonize NFκB. This study investigates the polymorphism-dependent influence of the PPAR-γ agonist pioglytazone (P) on NFκB expression on CD40+ peripheral blood lymphocytes.

METHODS: Peripheral blood mononuclear cells (MNC) from 6 volunteers were cultured 24 hours in 10, 30 and 100 μmol P diluted in dimethylformamide versus phosphate buffered saline (PBS) and pure dimethylformamide as controls and then analyzed by flow cytometry (Coulter EPIX) with anti-CD40-FITC (Beckman Coulter) and anti-NFκB-RPE (Becton Dickinson). The polymorphism Pro12Ala of PPAR-γ2 was assessed by PCR with restriction analysis.

RESULTS: Expression of CD40 cells was unchanged in control or P cultures. Expression of NFκB in CD40-positive cells significantly increased in all control cultures after 24 hours incubation. Addition of P to MNC induced from 5 to 25% dose-dependent decrease in NFκB expression. The impact of P on CD40+ cells with the Pro12Ala polymorphism of PPAR-γ was greatest on MNC from patients bearing the Ala12 allele.

CONCLUSIONS: CD40-positive MNC are susceptible to activation of PPAR-γ by P, which decreases NFκB expression in a dose-dependent manner. Reduced NFκB expression depends on the polymorphism Pro12Ala of the PPAR-γ. PPAR-γ may influence immunoglobulin isotype switching, PPAR-γ agonists perhaps having a role in modulation of allergic inflammation.

344 Gene Expression Profiling Reveals that Hydrocortisone Promotes B-cell Proliferation and Differentiation

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RATIONALE: Immunoglobulin E (IgE) is essential for mediating allergic diseases. Induction of IgE synthesis in human B-lymphocytes requires co-stimulation of CD40L and cytokine IL-4. Studies have shown that hydrocortisone (HC) can synergize with IL-4 to induce IgE synthesis in human B cells. However, the mechanism of HC stimulation and the specific functions that HC induces in B cells are not known. We wanted to determine the specific roles that HC plays in B-cell activation and IgE production.

METHODS: Human tonsillar B-cells were purified and stimulated with anti-CD40 + IL-4 or anti-CD40 + IL-4 + HC for 24 hours with non-stimulated B-cells as controls. RNA was extracted and hybridized to Affymetrix Genechip Array. Microarray raw data was then imported to *FlexArray* software and subjected to normalization and statistical testing. Differentially expressed genes were selected for gene ontology and pathway analysis using Ingenuity Pathway Analysis (IPA) software.

RESULTS: 49 unique differentially expressed genes were identified compared to stimulation with or without HC. The majority of these genes were involved in cellular growth and proliferation, such as markedly up-regulated IL7R and CXCR4. Surprisingly, pathway analysis revealed candidate genes in the Axonal-guidance signalling pathway, which was significantly altered upon HC stimulation. This included nervous system related genes EFHB2, CXCR4 and WntB5.

CONCLUSIONS: HC promotes B-lymphocyte growth and proliferation when addition to IL-4, but at 24 hours does not specifically activate novel genes related to IgM to IgE class switching. In addition, HC stimulates pathways involved in nerve system development. This suggests that HC may play a role in B-cell trafficking, migration and follicular organization

345 The Role of BTK in TLR Signaling

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RATIONALE: Bruton's tyrosine kinase (Btk) has recently been demonstrated to play an important role in signaling pathways of multiple toll-like receptors (TLR). In this study we examined the effect of Btk deficiency in X-linked agammaglobulinemia (XLA) patients on TLR signaling, and the role that Btk might play in normal controls and common variable immunodeficiency (CVID) patients with known TLR defects.

METHODS: B cells from normal patients were stimulated with TLR7, 7/8 and 9 ligands in the presence or absence of LFM-A13, a Btk-family antagonist, and B cell maturation was assessed by looking for production of activation induced deaminase (AID). TLR activity in neutrophils was assessed using a novel assay for TLR signaling dysfunction in which neutrophils are stimulated with TLR agonists and CD62L shedding is assessed by flow cytometry. This was done using fresh blood from XLA patients, CVID patients, and normal controls in the presence or absence of LFM-A13.

RESULTS: In normal B cells TLR-induced expression of AID is completely blocked when cells are treated with LFM-A13. TLR induced CD62L shedding was not affected in XLA patients, CVID patients or normal controls treated with the BTK inhibitor.

CONCLUSIONS: Btk is necessary for TLR induced activation of B cells. While the literature has pointed to the importance of Btk in TLR induced inflammation and XLA patient cells have demonstrated decreased inflammatory response when stimulated with TLR ligands, this novel neutrophil assay for defects in TLR signaling is not useful in assessing possible TLR dysfunction due to Btk deficiency.

346 ADAM-10 Overexpression Inhibits B cell Development and Promotes Myeloid-derived Suppressor Cell Granulopoesis

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RATIONALE: The binding of IgE to its low affinity receptor, CD23, serves as a negative feedback signal to inhibit further IgE production. However, catabolic cleavage of CD23 from the surface of B cells by its primary 'sheddase', a disintegrin and metalloprotease 10 (ADAM10) may interrupt this signal. In lieu of ADAM10$^{-/-}$ mice which die *in utero*, we generated transgenic (TG) mice that overexpress ADAM10 on B cells to determine the role of ADAM-10 in B cell development, CD23 cleavage, IgE production, and allergic airway inflammation.

METHODS: ADAM-10 overexpression was directed to the B cell lineage with use of the H-2Kb promoter and the IgH enhancer. Lymphocyte development, immunoglobulin production, and cytokine profiles were analyzed via flow cytometry, ELISA, and multiplex cytokine assays, respectively. Functional assays and cellular imaging were completed following fluorescence-activated cell sorting (FACS). Statistical significance between groups of more than 3 mice was determined using appropriate tests ($p < .01$).

RESULTS: Surprisingly, ADAM-10 overexpression dramatically impaired B cell development and promoted massive production of circulating myeloid-derived suppressor cells (MDSCs, CD11b + Gr-1+) implicated in suppressing anti-tumor immune responses. Interestingly, ADAM-10 TG mice have elevated levels of vascular endothelial growth factor (VEGF). This supports other reports identifying VEGF as an inhibitor of B cell development and a mediator of MDSC production in tumor models.

CONCLUSIONS: Therefore, anti-VEGF immunotherapy may hinder tumor evasion by preventing the development of MDSCs. This finding also indicates that ADAM-10 may play a critical role in the release of VEGF from bone marrow progenitors, implicating ADAM-10 as a regulator of hematopoiesis and angiogenesis.