314 Abstracts

Patient Perceptions of an Inhaled Asthma Medication Administered as an Inhalation Powder via the Diskus™ or as an Aerosol via a Metered Dose Inhaler

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The purpose of this study was to compare patient preference, ease of use, and correctness of use of fluticasone propionate (FP) inhalation powder administered via the Diskus™ and FP inhalation aerosol administered via MDI. In 154 adult patients with asthma and a limited history of MDI use, the Diskus™ and the MDI were compared in a randomized, open-label, 3-week cross-over study. Following the use of both devices, more patients perceived the Diskus™ easier to use (59%) and preferred it overall (60%) compared with the MDI (P<0.016). Ninety-eight percent of the patients were able to correctly perform all the maneuvers necessary to use the MDI vs. 91% for the Diskus™ following a single demonstration and/or reading the instructions. Compliance was significantly better for the Diskus™ with 95.2% of patients using the Diskus™ as directed compared to 89.7% for the MDI (P=0.021). If given the choice 59% of patients indicated that they would most likely request the Diskus™ from their physician (P=0.025). Conclusion: Despite the fact all patients had prior, albeit minimal, experience using an MDI, the Diskus™ was used correctly by nearly all patients (91%) following minimal instructions. Compliance rates were significantly higher with the Diskus™ compared to the MDI. In addition, most patients indicated they would likely request the Diskus™ from their physicians indicating a high level of acceptance of the Diskus™ in this patient population.

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315 Nebulized Combined Formoterol and Budesonide (NCFB) for Children Younger than 5 Years of Age with Persistent Asthma

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RATIONALE: NAEPP 2002 updated guidelines stated that the preferred treatment for all chronic moderate and severe persistent asthmatics is the combination therapy utilizing inhaled long acting beta2-agonists and inhaled corticosteroids. However, at the present time the only commercially available combination medication is fluticasone/salmeterol dry powder inhaler (DPI), where some children under 5 years of age are unable to master the inhalation technique to achieve the full benefits of this combination medication.

METHODS: We report the first use of NCFB, by dissolving formoterol 12 mcg powder from commercially available Foradil capsule into budesonide 0.25 mg/2 ml or 0.5 mg/2 ml (Pulmicort respules). A trial of NCFB was given to three male children (average age 3.75 years old with persistent asthma who failed to respond adequately to various controller agents; salmeterol/fluticasone MDI with holding chamber and face mask, fluticasone and salmeterol combination DPI, montelukast and nebulized budesonide. However, at the present time the only commercially available combination medication is fluticasone/salmeterol dry powder inhaler (DPI), where some children under 5 years of age are unable to master the inhalation technique to achieve the full benefits of this combination medication.

RESULTS: For all three children, six weeks post NCFB therapy resulted in total reduction of hospitalizations (from two to none), emergency room visits (from three to none), rescue nebulized albuterol needed at night from seven to none and short burst oral prednisone (from six to none) in comparison with six weeks prior starting NCFB regimen.

CONCLUSIONS: Nebulized combined formoterol with its fast and long acting bronchodilatory profile, in combination with budesonide, a topical anti-inflammatory agent achieved rapid reduction of asthma symptoms and decreased use of rescue medications, emergency room visits and hospitalizations.

Funding: Case study

316 Infliximab Therapy for Severe Steroid-Dependent Asthma and COPD: A Case Report

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BACKGROUND: In select cases of severe asthma, daily or alternate day oral corticosteroids are required to provide symptomatic control. Elevated levels of tumor necrosis factor-alpha (TNF-α) have been found in the sputum of asthmatics. TNF-α can increase bronchial hyperreactivity, upregulate adhesion molecules, facilitate immigration of inflammatory cells and activate pro-fibrotic processes. Infliximab, a monoclonal antibody directed against TNF-α, has improved lung function in rheumatoid arthritis with concurrent asthma. It has proved beneficial in certain inflammatory diseases recalcitrant to systemic corticosteroids suggesting that TNF-α may contribute to corticosteroid resistance.

CASE SUMMARY: We report a 53-year-old female, former smoker, with severe persistent asthma (FEV1 reversibility 11-67%) and moderate COPD requiring daily or alternate day oral corticosteroids for the past 5 years. Her stable prednisone dose was 10 mg daily. Because of increased asthma symptoms this dosage was increased to 20 mg/day prior to infusion. Three doses of infliximab (5 mg/kg) were administered at week 0, week 2 and week 6. Transient dizziness was observed only during the first infusion. Prednisone was tapered to 10 mg every other day. Baseline FEV1 values improved from 33% to a maximum of 66% (week 4). However, 12 weeks after the third infusion (week 18), FEV1 values precipitously regressed towards baseline requiring an extended oral prednisone burst.

DISCUSSION: To our knowledge, this is the first reported case of a steroid-dependent asthmatic with COPD who has responded to infliximab. A longer trial is planned to determine if the magnitude of improvements can be augmented and sustained.

Funding: Centocor

317 A Direct Interaction Between Clara Cell Secretory 10 KD Protein (CC10) and Inflammatory Cell Types

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RATIONALE: The importance of Th2-associated inflammation in the expression of bronchial hypersensitivity has been established, and significantly altered pulmonary Th2-associated allergic inflammation is seen in CC10-deficient mice (Li et al, Cutting Edge, J Immunol 2001). But, the interaction of CC10 with inflammatory cells remains to be defined.

METHODS: To this end, affinity cross-linking experiments were performed using recombinant human CC10 (rCC10) and various cell lines, including Jurkat, HMC-1 and EL-4. The cells were incubated with non-reduced or reduced 125I-rCC10 in the absence or presence of excess unlabeled rCC10. The cells were then incubated further with 0.2 mM DSS and lysed. The supernatants were resolved by gel electrophoresis. Inflammatory cytokine/chemokine gene “Superarray” was used to investigate modulation of gene expression in ionomycin/PMA-activated, rCC10 (100 ng/ml)-treated Jurkat and EL-4.

RESULTS: Affinity cross-linking experiments by incubating the reduced or non-reduced form of 125I-rCC10 showed a distinct 190-kD band, and this 190-kD band was undetectable when 1 μM unlabelled rCC10 was added to the cells prior to 125I-rCC10 binding and affinity cross-linking. As expected, no protein band was detected in the absence of DSS. Furthermore, significant changes in the profile of chemokine/chemokine receptor expression was found in “Superarray” analysis of activated Jurkat and EL-4 treated with rCC10.

CONCLUSIONS: These results suggest that rCC10 binds to the surface of inflammatory cell types with specificity, and that the activities of CC10 are receptor-mediated. Further molecular characterization of the CC10 binding protein (receptor) may provide insight into its molecular mechanism.

Funding: Research Management Group

318 Functional Analysis of the Chemokine Receptor CCR3 on Airway Epithelial Cells

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RATIONALE: Chemokine receptors have been recently identified on structural cells, although little is known about their function. We previously reported the expression of a functional CCR3 receptor on airway epithe-
lial cells (J Immunol 2001;166:1457). Since epithelial cells are an important source of CCR3 ligands such as eotaxin, MCP-4, and RANTES, we speculated that epithelial-bound CCR3 may regulate epithelial functions.

**METHODS:** We challenged the human airway epithelial cell line BEAS-2B with eotaxin to determine the effect on the expression of chemokine and chemokine receptor genes using microarray technology.

**RESULTS:** Total RNA was extracted from cells stimulated for 18 hr with medium or eotaxin, 10 and 100 ng/ml. Labeled cDNA was hybridized to GE Array superarrays containing most chemokine and chemokine receptor genes. The following genes showed a >2-fold increase over media alone (n=2-3) in cells stimulated with 100 ng/ml eotaxin: CCR3, XCR1, HCC-4, TARC, eotaxin, SDF1, NAP2, IL-8 and MIG. Array results were validated by real-time PCR for CCR3 (n=6), eotaxin (n=2), SDF-1 (n=1) and IL-8 mRNA (n=1) and by ELISA for IL-8 (n=2). We screened unstimulated BEAS2B cells by FACS analysis with antibodies directed against CCR1-10 and CXCR1-5 and found the following receptors expressed: CCR5>CXCR4>CCR9>CCR3>CXCR5>CCR6. Upregulation of CCR3 surface expression by eotaxin was confirmed by FACS in primary bronchial epithelial cells (n=3), but not in BEAS-2B cells (n=1).

**CONCLUSIONS:** Changes in gene expression after eotaxin challenge of epithelial cells suggest a potential role of epithelial CCR3 in driving regulatory pathways in the mucosal chemokine network.

*Funding: AstraZeneca*

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**319 Soluble Recombinant Human Interleukin-4 Receptor (rhIL-4R) Inhibition of T Lymphocytes from Antigen-Specific Cell Lines and Segmental Allergen Challenges**

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**RATIONALE:** Interleukin (IL)-4 has important activities that contribute to asthma and allergies including the differentiation of naive T cells into Th2-like lymphocytes, inhibition of apoptosis of established Th2 cells, and induction of corticosteroid resistance by these cells. Soluble rhIL-4R retains the ability to bind IL-4 with high affinity and acts as an IL-4 antagonist. These studies were performed to identify anti-inflammatory effects of rhIL-4R on T lymphocytes.

**METHODS:** Tetanus-specific T cell lines were grown using tetanus and IL-2 and weekly addition of irradiated, autologous antigen-presenting cells and fresh antigen. After 3 weeks, cell lines were cultured with or without rhIL-4R. In parallel experiments, CD3+ T lymphocytes were obtained via negative affinity selection from subjects who underwent segmental allergen challenges (SAC) and were then cultured with or without rhIL-4R.

**RESULTS:** RhIL-4R significantly inhibited proliferation (49.0 ± 0.4%; p<0.01) of tetanus-specific T cell lines but did not influence apoptosis as measured by expression of annexin V nor did it modulate dexamethasone-mediated inhibition of proliferation. Soluble IL-4R was associated with 35.0 ± 0.5% inhibition of proliferation of SAC-derived T lymphocytes. No difference was observed in apoptosis. However, the presence of rhIL-4R did significantly down-regulate expression of the anti-apoptotic protein Bcl-2 (67.3 ± 5.2%). RhIL-4R significantly inhibited secretion of cytokines associated with Th2-like lymphocytes (IL-5 and IL-13) without modulating production of the Th1-derived cytokine IFN-γ. In contrast, rhIL-4R dramatically upregulated production of the anti-inflammatory cytokines IL-10 and TGF-β.

**CONCLUSIONS:** Attenuation of T cell responses to allergens may contribute to the clinical efficacy of rhIL-4R observed in asthmatic subjects.

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**320 Human B Lymphocytes Produce IL-13: Role As an Autocrine Cytokine in IgE Synthesis**

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**RATIONALE:** Our laboratory has demonstrated that human B lymphocytes are capable of producing IL-13. This study addressed the secretion of IL-13 by B cells and its role in IgE synthesis.

**METHODS:** Human B lymphocytes were isolated from tonsils and purified by rosetting with sheep rbc or by positive or negative selection using MACS. B cells were stimulated with anti-CD40 antibodies with and without IL-4. Supernatants were harvested and IL-13 and IgE were measured by ELISA.

**RESULTS:** IL-13 protein was detected from human tonsillar B cells first on day 3, and accumulated through day 7 of culture. Using ELISA spot assays, we demonstrated that 1-5% of cultured B cells secrete IL-13. The synthesis of IL-13 required both CD40 and IL-4 stimulation, as there was no detectable IL-13 when B cells were cultured with either stimulus alone. B cells prepared by sheep rbc depletion (<2% T cells) and B cells purified by positive selection (CD19+ MACS beads, <0.5% T cells) produced between 15-50 pg/ml of IL-13. We assessed potential contaminating cells; neither purified T cells (10^6/ml) nor follicular dendritic cells (10^6/ml) cultured with CD40 and IL-4 produced IL-13. Addition of IL-13 neutralizing antibodies to purified B cell cultures, but not anti-CD3 Ab or the isotype control, inhibited IgE production by up to 80% (n=4, Figure 4), and diminished IgE (Ce) transcripts by 50%.

**CONCLUSIONS:** Human B lymphocytes produce significant IL-13 and neutralization of IL-13 impairs IgE synthesis. IL-13 may be an important autocrine growth factor for IgE producing B lymphocytes.

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**321 Molecular Mechanism of IL-5 Receptor Proteasome Degradation**

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The IL-5 receptor (IL-5R) consists of a ligand specific alpha chain (IL-5R alpha) and a shared signaling component, betaC. My previous studies revealed that betaC receptor signaling is down-regulated by ubiquitin/proteasome degradation of its cytoplasmic domain, followed by endocytosis and lysosomal degradation of the remaining truncated IL-5R complex.

**RATIONALE:** Since the molecular mechanisms that initiate and mediate betaC proteasome degradation are not defined, we examined the role of betaC tyrosine/serine phosphorylation and ubiquitination in its proteasome degradation. Moreover, the mechanism by which betaC is targeted to the proteasomes was also investigated.

**METHODS:** Kinase inhibitors were used to treat TF-1 cells and assayed for their ability to inhibit betaC ubiquitination and proteasome degradation. In addition, two betaC mutants, one mutated in all 6 critical cytoplasmic tyrosine residues (Y-F6) and one mutated on Ser 585 (S-G), were co-transfected with IL-5Rα into COS cells and evaluated for their ability to prevent betaC ubiquitination and proteasome degradation. Co-immunoprecipitation assays were also performed to determine if betaC associated with valosin-containing protein (VCP), a proteasome-targeting factor.

**RESULTS:** Treatment of TF-1 cells with tyrosine kinase inhibitors decreased betaC ubiquitination and proteasome degradation by more than 50%, as compared to untreated cells. The transfected betaC mutants confirmed the data seen with the kinase inhibitors. Moreover, protein/protein interactions were detected between betaC and VCP.

**CONCLUSIONS:** BetaC phosphorylation is required and precedes its ubiquitination and proteasome degradation of its cytoplasmic domain. These results suggest that betaC phosphorylation may be the initiating signal for its signal termination by the proteasomes.

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