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340 Phenotypic Profiling of Blood and Airway Secretions in Asthmatic and Healthy Subjects

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INTRODUCTION: We have extended the microvolume laser scanning cytometry platform, SurroScan™, to enable analysis of inflammatory markers in

airway secretions. In previous studies the method has been used to identify, characterize, and enumerate hundreds of unique cell populations from less than 2ml of blood. Here we have applied the technique to blood, induced sputum (IS), bronchoalveolar wash (BW), and bronchoalveolar lavage (BAL) to preliminarily identify inflammatory markers in these compartments that might best reflect the fundamental pathophysiology or the clinical outcomes of asthma.

METHODS: Ten asthmatic (FEV1 >50%, PC20-M <8 mg/ml) and ten healthy volunteers underwent venipuncture and sputum induction on one day, and returned within two weeks to undergo bronchoscopy for BW (50ml of warmed normal saline instilled and aspirated) and BAL (100ml instilled and aspirated). The cytometry panel consisted of 64 three-color assays, directed against >50 antigens on blood cells, and 32 three-color assays, directed against >35 antigens on cells in airway secretions. While whole blood samples were analyzed at room temperature with homogenous (no washing) assays, we found that airway secretions are best processed on ice with washing to remove excess antibody and reduce non-specific binding. The assay panel includes markers important in activation, inflammation and adhesion including CD69, HLA class II, CD44, CD54 (ICAM-1), CD58 (LFA3), and CD62L (L selectin).

RESULTS: Our methods successfully identified specific cell populations in samples from airway secretions. In BAL and IS we were able to identify populations of granulocytes, including eosinophils, lymphocytes, including T cells and B cells, as well as non-leukocyte populations. In these samples, an autofluorescent macrophage population that expresses CD45, CD16, HLA-DR, HLA-DQ, CD86 and low levels of CD14 is prominent. In comparing samples from asthmatic and healthy subjects, we have found that the number of eosinophils as a percentage of total granulocytes is higher in IS and blood of asthmatic subjects. In addition, our preliminary data indicate lower numbers of $\gamma\delta$ T cells and greater numbers of NK cells and eosinophils in blood from

asthmatic subjects. We are currently analyzing expression of other activation and inflammatory markers.

CONCLUSION: We have extended the SurroScan™ cytometry platform to analyze local sites of disease manifestation: induced sputum, bronchoalveolar wash, and bronchoalveolar lavage. The methodology can help identify markers more closely related to disease severity, control, and treatment. A key application will be evaluation of the local and systemic effects of exacerbants and treatments of asthma and of other airway diseases.