Toxicity and Pharmacodynamics of Cy317HuXCy, a Surrogate for AMG 317, in Cynomolgus Monkeys: 4-Week and 13-Week Repeated-Dose Studies

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Rationale: AMG 317 is a fully human monoclonal antibody undergoing evaluation as an asthma treatment based on its ability to bind to IL-4Rα. Because AMG 317 binds only to human and chimpanzee IL-4Rα, a surrogate antibody (Cy317HuXCy) was used to assess toxicity in cynomolgus monkeys.

Methods: Two studies were conducted. The first was a 4-week repeated-dose study of placebo or Cy317HuXCy (5, 20, or 100 mg/kg) (10 animals/group) administered once weekly by subcutaneous injection. The second was a 13-week repeated-dose study evaluating once-weekly administration of placebo, the same subcutaneous doses as in the first study, and an intravenous dose (100 mg/kg) (10 animals in the 0 and 100 mg/kg groups; 6 animals in the 5 and 20 mg/kg groups). In both studies, the treatment period was followed by a 12-week recovery period.

Results: All animals survived to the scheduled necropsies. No remarkable findings were observed on clinical, ophthalmologic, or cardiovascular examination, food consumption, body weight, clinical pathology, histopathology, peripheral blood immunophenotyping, organ weights, or macroscopic observations at necropsy. The 4-week study included an examination of sequential serum samples to determine if circulating levels of IgE, sCD23, sVCAM-1, LTC-4, MMP-9, 15(S)HETE, and SCCA-1 were altered by administration of Cy317HuXCy. No clear effects were observed on these pharmacodynamic markers. Minor, generally reversible, microscopic findings were noted at the injection sites with 13 weeks of dosing, indicating an inflammatory response to Cy317HuXCy.

Conclusions: Weekly administration of Cy317HuXCy, a surrogate for the clinical candidate AMG 317, was well tolerated in 4-week and 13-week repeated-dose studies in cynomolgus monkeys.

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Elevated Levels of C-C Chemokines in Bronchoalveolar Lavage Fluid Are Predictive of P Outcome of Idiopathic Pulmonary Fibrosis

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Background: C-C chemokines play important roles in the pathogenesis of various interstitial l diseases, such as idiopathic pulmonary fibrosis (IPF). Although elevated C-C chemokine levels h been revealed in bronchoalveolar lavage (BAL) fluid of patients with IPF, it remains to be determi whether they influence the patient outcome.

Method: We evaluated the levels of four C-C chemokines, monocyte chemoattractant protei (MCP-1/CCL2), macrophage inflammatory protein-1a (MIP-1a/CCL3), thymus and activati regulated chemokine (TARC/CCL17), and macrophage-derived chemokine (MDC/CCL22), in B fluid of 39 patients with IPF, comparing those alive 5 years after diagnosis and those deceased. It also examined whether serum markers are predictive of the patient outcome and correlated with chemokine levels in BAL fluid.

Results: Among 39 patients (29 males, age: 62.8±1.4 years), 19 patients (48%) died within 5 ye after the diagnosis. %VC of surviving and non-surviving patients were 74.8 ± 4.3 and 72.7 ± respectively. Median CCL2 levels of surviving and non-surviving patients were 154.3 (IQR: 67 381.8) and 427.2 (IQR: 329.2 - 1184.1) pg/mL, respectively (p<0.02). CCL3 levels in BAL fluid did differ between the surviving and non-surviving patients. CCL17 was detected in BAL fluid of 6 (32 out of 19 patients who was deceased within 5 years after the diagnosis, whereas it was detectable o in one (5%) of 20 surviving patients (p<0.05). CCL22 was detectable in BAL fluid of 9 (47%) ou 19 patients who was deceased within 5 years after the diagnosis, whereas it was detectable only in (5%) of 20 surviving patients (p<0.01). *Conclusion:* Elevated levels of CCL2, CCL17, and CCL22 BAL fluid might be predictive of poor outcome of the patients with IPF.

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CCL17 and CCL22 as a Pathogenesis of Pulmonary Fibrosis

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RATIONALE: We have previously demonstrated that Th2 chemokines, CCL22/MDC and CCL17/TARC were selectively induced in a rat model of radiation pneumonitis(Inoue T, et al. ERJ 2004;24:49). We also found elevated levels of CCL22 in bronchoalveolar lavage fluids (BALF) from patients with idiopathic pulmonary fibrosis (IPF), but the role of CCL17 in pulmonary fibrosis was not clarified sufficiently.

PURPOSE: To further elucidate the pathophysiological role of Th2 chemokines in pulmonary fibrosis, we examined CCL17 and CCL22 in a mice model of bleomycin-induced pulmonary fibrosis.

METHODS: C57BL/6 mice were intratracheally administered with 100 µl of either bleomycin solution (3mg/kg, BLM group) or phosphate buffered saline (CTL group). We sacrificed mice at day 7, 14, 21 and determined the levels of CCL17 and CCL22 in the BALF and lung tissue homogenates with ELISAs specific for CCL17 and CCL22, respectively.

RESULTS: CCL17 in lung homogenates was significantly higher in BLM group than that in CTL group at day 7 (4656±353 vs. 2753 ±356, p<0.05), 14 (2985±123 vs. 2287±198, p<0.05) and 21 (3307±265 vs. 2183±180, p<0.05). CCL17 in BALF was also significantly higher in BLM group than that in CTL group at day 7 (292.2±30.2 vs. 18.9±2.1, p<0.01), 14 (210.9±26.1 vs. 30.0 ±7.3, p<0.01) and 21(90.7±5.5 vs 28.1±3.0, p<0.01). In contrast, there was no significant difference in CCL22 of lung homogenates and BAL fluids between the two groups at any time points.

CONCLUSIONS: The involvement of Th2 chemokines was confirmed in a mouse model of bleomycin-induced pulmonary fibrosis. However, in contrast to a rat model of radiation pneumonitis or human IPF, CCL17 was predominantly elevated in this model, suggesting disparate regulation of Th2 chemokines among species or injuries.

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A Novel Mechanism of Neutrophil Recruitment Involving Proteolytic Cleavage of Collagen

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Background: Neutrophils are important inflammatory mediators of a variety of chronic l diseases, such as CF and COPD. While specific chemokines have been found to play a role in th conditions, our group has recently determined a tripeptide (proline-glycine-proline (PGP)) is important mediator of neutrophil influx in animal models of lung inflammation. Although suspected be extracellular matrix (ECM)-derived, the mechanism of the generation of this peptide is unknown

Hypothesis: PGP is derived from collagen via proteolytic cleavage.

Results: Utilizing CF as a disease model, we determined that PGP was found in elevated quanti in sputum from CF individuals. When CF sputum was incuhated with both type I or type II collagen vivo, there was a significant increase in PGP generation. We examined which proteases were found CF sputum, noting elevated levels of matrix metalloprotease (MMP)-8, -9, -12, and human neutro elastase (HNE). We also determined the presence of a novel serine protease, prolyl endopeptid (PE), in CF sputum. When we compared the correlation between the activity of these proteases w PGP generation in CF sputum, we found notable correlation between MMP-9 activity with PGP PE activity with PGP. We then instilled recombinant proteases, alone or in combination, into m intratracheally and then bronchoalveolar lavage (BAL) afterwards. PGP generation was not noted w enzyme alone but the combination of MMP-8+PE and MMP-9+PE generated PGP. Finally, we show an attenuation of this generation with the use of specific MMP-8, MMP-9 and PE inhibitors.

Conclusions: MMP-8,-9 and PE seem, in combination, to have the capability of cleaving colla to generate PGP.

Clinical Implications: Inhibition of this pathway of inflammation may yield novel therapeutic the treatment of dysregulated neutrophil inflammation in chronic lung disease.

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Chemokine Receptor 3 (Ccr3) Plays an Important Role In Allergic Rhinitis Induced by Toluene Diisocyanate

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Rhinitis often precedes and is a co-existing disease in workers with occupational asthma. Therefore, understanding occupational rhinitis may result in new diagnostic and therapeutic strategies that may help prevent the development of asthma. We previously developed a murine model of toluene diisocyanate (TDI) rhinitis displaying increased expression of IL-4, IL-5, IL-13, IFNγ, TNFα and IL-1β as well as extensive eosinophil infiltration into the nasal mucosa. Whole-genome expression analysis demonstrated that genes involved in inflammatory cell recruitment/movement and, specifically, chemotaxis, chemokine activation and chemokine receptor binding were differentially expressed in the nasal mucosa of mice with TDI rhinitis. The chemokine receptor 3 (Ccr3) pathway is important for the recruitment of eosinophils and Th2 lymphocytes to sites of allergic inflammation. We hypothesized that the Ccr3:ligand axis is activated in TDI rhinitis leading to the recruitment of Th2 lymphocytes and eosinophils. Mice were exposed to 50 ppb TDI vapor via inhalation for 12 consecutive weekdays and real-time PCR was used to analyze the expression of chemokine, chemokine receptor and immune genes in the nasal mucosa 24 hours following the final exposure. Expression of Ccr3 and its ligands, Ccl11 (eotaxin I) and Ccl24 (eotaxin II) were upregulated in the nasal mucosa following TDI inhalation. Administration of neutralizing anti-Ccr3 antibodies prior to TDI exposure prevented the upregulation of Ccl11 and Ccl24. In addition, neutralization of Ccr3 markedly reduced TDI-induced changes in IL-4, IL-5, IL-13, TNFα and IL-1β expression. These results support the hypothesis that Ccr3 is an important mediator of inflammation in TDI rhinitis.

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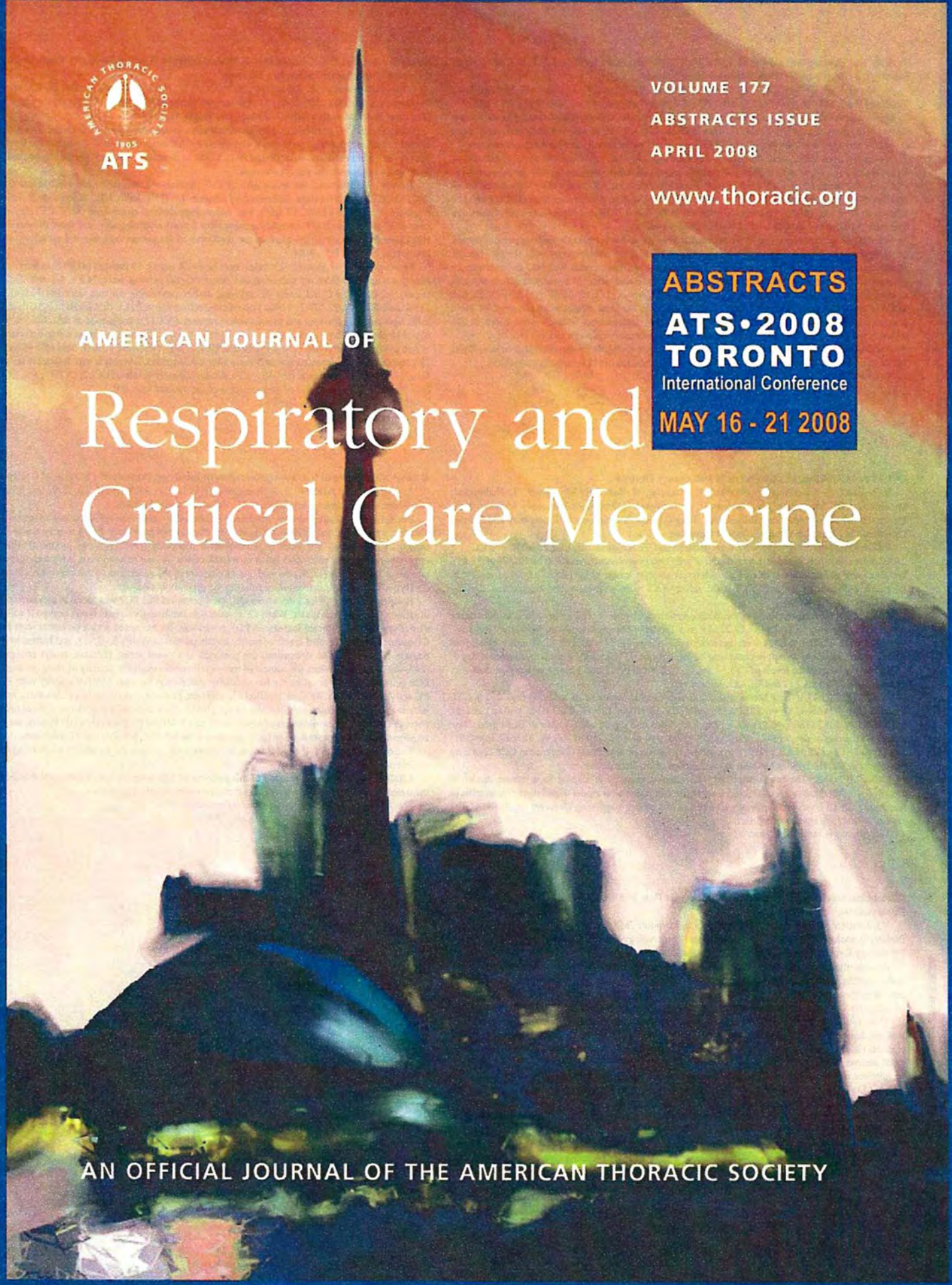

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