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Full Length And Processed Forms of IL-33 Are Bioactive And Can Be Rapidly Released *In Vivo*

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Purpose: IL-33 is an IL-1 family cytokine that can initiate inflammation and is a potential therapeutic target for asthma and related diseases. IL-33, like IL-1α is processed during release from necrotic cells. The mechanism and biological function of processing of IL-33 remains poorly understood. Unlike other IL-1 family cytokines processing of IL-33 by caspase abolishes its bioactivity. Recent *in vitro* studies using an *in vitro* translated full length (FL) IL-33 suggest N-terminal processing by the inflammatory proteases neutrophil elastase (NE) and cathepsin G (CG) can increase the bioactivity of IL-33[1]. Here we have investigated IL-33 processing and bioactivity in an *Alternaria alternata* (ALT) challenge model of lung inflammation and in mouse and human *in vitro* systems.

Methods: Wild type (WT), IL-33$^{-/-}$ and ST2$^{-/-}$ BALB/c mice were challenged by a single intranasal (i.n.) dose of ALT extract (25 μg), recombinant mouse FL IL-33 lysate (amino acids 1-266) or mature IL-33 (109-266). Bronchoalveolar lavage (BAL) was analysed by ELISA and western blot and airway resistance was measured. Mouse bone marrow derived mast cells were stimulated with BAL, recombinant mouse FL or mature IL-33 and IL-6 release measured. Human recombinant FL IL-33 lysate (1-270) and mature IL-33 (112-270), with or without NE and CG treatment, and human lung lysate were analysed by western blot. HUVECs were stimulated with FL, mature or NE and CG *in vitro* processed forms of IL-33 and NFκB signalling and IL-6 release measured.

Results: Full length (30 kDa) and processed forms (~18 kDa) IL-33 were released into bronchioalveolar lavage (BAL) 5 min after ALT challenge. Highest levels of IL-33 were measured 30 min after ALT challenge (13.5 ± 5.4 ng/ml, n=4). BAL contained predominantly processed IL-33 and could stimulate IL-6 release from WT but not IL-33$^{-/-}$ mouse mast cells *in vitro*. Recombinant mouse FL and mature IL-33 were bioactive in mouse mast cells (EC_{50} 356 pM 95% CI 164-772 pM and 406 pM 95% CI 150-1095 pM respectively, n=4) and FL IL-33 induced airway resistance in WT ($p<0.01$ for FL IL-33 v control, n=4) but not ST2$^{-/-}$ mice ($p<0.001$ for wild type v ST2$^{-/-}$ mice, n=4). Similar to results in mice both recombinant human FL and mature IL-33 were bioactive and could stimulate NFκB signalling (EC_{50} 43 pM 95% CI 11-106 pM and 34 pM 95% CI 11-163 pM respectively, n=4) and IL-6 release (EC_{50} 391 pM 95% CI 260-586 pM and 485 pM 95% CI 292-806 pM respectively, n=4) in HUVECs. Treatment of human FL IL-33 with NE or CG *in vitro* generated ~20-21 kDa forms that have similar molecular weights to endogenous forms of IL-33 detected in human lung. Treatment of human FL IL-33 with NE or CG increased the potency of IL-33 in both HUVEC NFκB signalling (3.7 and 3.1 fold respectively, n=2) and IL-6 release assays (2.9 and 2.8 fold respectively, n=2).

Summary: FL and processed forms of IL-33 are detected in a model of pulmonary inflammation in mice and in human lung. IL-33 appears to resemble IL-1α in being

bioactive in FL and mature forms with serine proteases such as NE and CG able to process full length cytokine to mature forms with increased bioactivity.

References:

1. LeFrançais et al Proc Natl Acad Sci USA 109: 5, 2012