ImmunoTools *special* Award 2015

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Coordinate regulation of eosinopoiesis and neutropoiesis through NO and 5-lipoxygenase: effects of cytokines

- **Background**. Eosinophils and neutrophils are closely related granulocyte lineages, known for their roles in allergy and infection, respectively[1,2]. Both lineages are produced in the bone-marrow, under the influence of hemopoietic cytokines, inflammatory mediators and immunomodulatory drugs. Eosinopoiesis in mouse bone-marrow is upregulated by glucocorticoid stress hormones[3,4], and by proallergic mediators, including cytokines[5] (eotaxin, IL-13, IL-9, IL-33), and suppressed by antiallergic drugs, including several inhibitors of the 5-lipoxygenase (5-LO) pathway[6,7]. 5-LO products, especially cysteinyl-leukotrienes (CysLT), enhance eosinopoiesis by blocking a proapoptotic mechanism that promotes NO production through inducible NO synthase (iNOS), and ultimately induces immature eosinophil apoptosis[8]. In addition, neutropoiesis in cultured mouse bone-marrow is upregulated by 5-LO products, especially Leukotriene (LT)B4. All-trans retinoic acid (ATRA) inhibits eosinopoiesis but enhances neutropoiesis in bone-marrow culture. The neutropoietic effect of ATRA is mediated by NO production, highlighting the ability of multilineage regulators to coordinately increase neutrophil production and suppress eosinophils by an effect on iNOS. Cytokines encompass functionally distinct classes that directly stimulate eosinopoiesis (IL-5)[9] or neutropoiesis (GM-CSF, G-CSF[10]), or modify either process (IL-13, eotaxin, IL-9, IL-33, for eosinopoiesis; IL-17A, TNF-a, for neutropoiesis). Cross-regulation of these two processes may involve cytokines acting to induce iNOS or 5-LO, ultimately signaling through NO or Leukotrienes.

- **Goal of the study:** to screen a wide panel of cytokines for the ability to cross-regulate eosinopoiesis and neutropoiesis, and to examine the roles of iNOS and 5-LO in mediating their cross-regulatory effects.

- **Study design**. Bone-marrow from wild-type (BALB/c, C57BL/6, PAS) or mutant mice of these backgrounds (lacking iNOS or 5-LO) will be cultured in the

presence of eosinopoietic or neutropoietic cytokines, alone or in synergistic combinations, and the production of eosinophils and neutrophils will be evaluated over a 7 day-period. Where indicated, the following recombinant murine cytokines will be examined for their ability to modulate either process, and to induce iNOS or 5-LO: Flt3L, G-CSF, IL-1alpha, IL-1beta, IL-3, IL-6, IL-9, IL-10, IL-11, IL-13, IL-16, IL-17A, IL-17C, IL-17E, IL-17F, IL-21, IL-22, IL-27, IL-31, IL-33, LIF, SCF, SDF-1a, SDF-1b. Their ability to counteract the effects of iNOS and NO through production of LTB4 and other leukotrienes in bone-marrow cultlure will be examined. Evidence for the involvement of iNOS or 5-LO will be obtained by comparing the appropriate mutant strains with their wild-type controls. This is a pilot study that concentrates on detection of effects in culture, because this design is sensitive, requires small cytokine amounts and a lesser number of animals, and can be better controlled than in vivo treatments.

- **Relevance**. Cross-regulation of the eosinophil and neutrophil lineages underlies fine tuning of the hemopoietic response to injury in the context of infection, allergy of drug treatment[11,12]. Results from this pilot study will help us to define whether a role for cytokine-mediated cross-regulation of these lineages can be demonstrated with the help of in vivo protocols.

- **References**. [1] *Immunol Rev.* 2011; 242:161-77 [2]*Annu Rev Pathol.* 2014;9:181-218. [3]*Br J Pharmacol*. 2004;143:541-48. [4]*Life Sci.* 2014;94:74-82. [5]*J Leukoc. Biol.* 2010;87885-9 [6]*Am J Respir Crit Care Med.* 2010; 181:429–37 [7]*Mediat Inflamm* Volume 2014, Article ID 403970 [8]*Nitric Oxide.* 2004;11:184-93 [9]*Am J Resp Cell Mol Biol* 1997, 17, 404-413 [10]*Life Sci* 2011. 88, 830-838 [11]*Life Sci* 2014, 94(1):74-82 [12]Xavier-Elsas et al. 2012**,** in *Eosinophils: Structure, Biological Properties and Role in Disease* (G. M. Walsh, Ed.), Cell Biol Res Progr. ISBN 978-1-61122-270-8, NOVA Pub., NY, pp. 203-220.