ImmunoTools IT-Box-139 Award 2013

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Basophils and their role in the immune network

Representing less than 1% of circulating leukocytes, basophils are one of the least abundant types of white blood cells. Nevertheless, high levels of FcεRI on their surface and IgE-mediated stimulation favor basophils being one of the most important cell types involved in allergic reactions and asthma. [1, 2]

Repeated exposure to allergens exacerbates allergic inflammation, but also bacterial infections were discussed to bear worsening influences to asthmatic patients. Most likely toll-like receptors (TLRs), key molecules in recognizing microbes and thus in innate immunity, trigger these asthma aggravating effects. [3, 4]

In human basophils TLR-2, 4, and 9 were identified but their potential to activate basophils upon stimulation with TLR-specific ligands is ambiguously discussed (TLR-2, 4) or unknown (TLR-9). For example lipopolysaccharide (LPS), a TLR-4 ligand, was shown to activate basophils of patients with IgG4-related disease but not in healthy donors. [5-8]

The aim of this study is to gain more detailed knowledge on the role of TLRs in the basophils of both healthy and allergic individuals.

To characterize basophil activation upon TLR stimulation, surface marker expression and cytokine release will be profiled. Therefore, purified human whole blood basophils of healthy, non-allergic donors will be stimulated with different concentrations of ligands to TLR-2, 4, and 9 (Pam3CSK4, LPS, and CPG-DNA) for several stimulation durations. Commonly used positive controls of basophil activation, namely αFcεRI antibody and formyl-methionyl-leucyl-phenylalanine, will be included in the study, too. As surface markers of basophil activation fluorescence dye labeled antibodies against molecules like CD63, CD203c, CD11a, CD11b, CD18, CD29 will be examined. Next to CD molecule expression, the secretion of cytokines IL-4, IL-6, IL-13, and IL-25 will be investigated.

The results of stimulated basophils of healthy donors will be compared to signaling in basophils of birch pollen and house dust mite allergic patients. They will be

stimulated with TLR ligands, positive controls, and additionally major birch pollen allergen Bet v 1a or major house dust mite allergens Der p 1 and Der p 2, respectively. Depending on the availability of birch allergic patients undergoing desensitization treatment, possible effects of the therapy will be investigated.

Literature:

1. Schneider, E., et al., Basophils: new players in the cytokine network. Eur Cytokine Netw, 2010. 21(3): p. 142-53.
2. Gauvreau, G.M., et al., Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. Am J Respir Crit Care Med, 2000. 161(5): p. 1473-8.
3. Micillo, E., et al., Respiratory infections and asthma. Allergy, 2000. 55 Suppl 61: p. 42-5.
4. Akira, S., Mammalian Toll-like receptors. Curr Opin Immunol, 2003. 15(1): p. 5-11.
5. Komiya, A., et al., Expression and function of toll-like receptors in human basophils. Int Arch Allergy Immunol, 2006. 140 Suppl 1: p. 23-7.
6. Watanabe, T., et al., Toll-like receptor activation in basophils contributes to the development of IgG4-related disease. J Gastroenterol, 2012.
7. Sabroe, I., et al., Toll-like receptor (TLR)2 and TLR4 in human peripheral blood granulocytes: a critical role for monocytes in leukocyte lipopolysaccharide responses. J Immunol, 2002. 168(9): p. 4701-10.
8. Bieneman, A.P., et al., Toll-like receptor 2 ligands activate human basophils for both IgE-dependent and IgE-independent secretion. J Allergy Clin Immunol, 2005. 115(2): p. 295-301.

ImmunoTools *IT-Box-139.3* for **Markus Steiner** includes 100 antibodies

FITC - conjugated anti-human CD1a, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD11a, CD11b, CD14, CD15, CD16, CD18, CD19, CD21, CD25, CD29, CD36, CD41a, CD43, CD45, CD45RA, CD46, CD52, CD53, CD54, CD58, CD62p, CD63, CD69, CD71, CD80, CD86, CD95, CD235a, HLA-ABC, HLA-DR, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE - conjugated anti-human CD2, CD3, CD4, CD8, CD11b, CD14, CD15, CD18, CD19, CD20, CD21, CD22, CD27, CD33, CD34, CD37, CD38, CD40, CD42b, CD45, CD45RB, CD50, CD72, CD95, CD105, CD147, CD177, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V

PE/Dy647 -tandem conjugated anti-human CD45

APC -conjugated anti-human CD3, CD4, CD7, CD8, CD10, CD11c, CD14, CD16, CD19, CD27, CD37, CD40, CD44, CD56, CD59, CD61, CD62L, CD62P, CD69, IL-6, Control-IgG1, Control-IgG2a, Control-IgG2b, Annexin V DETAILS

plus CD11a APC