ImmunoTools IT-Box-139 Award 2013

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The role of α_1-Antitrypsin in inflammation: effects on Neutrophil adhesion and Neutrophil extracellular traps (NETs) formation

Neutrophils are the main cells seen early in a response to infection or inflammation. To defend the host, neutrophils eliminate invading pathogens using different strategies like phagocytosis, degranulation (release of antimicrobial substances) and neutrophil extracellular traps (NETs), networks of extracellular fibers, primarily composed of DNA. During NETs formation neutrophils (similar to other cells of the innate immunity) expel decondensed chromatin, which is decorated with antimicrobial peptides and enzymes. NETs are meant to trap and to kill invading pathogens. In the framework of my PhD-thesis I am investigating the mechanism behind NETs formation and degradation, which is still poorly understood. Especially, I am focusing on characterizing NETosis in the presence of alpha1-antitrypsin (AAT), a major endogenous inhibitor of neutrophil elastase and suppressor of reactive oxygen species production, both of which are crucial for initiation of NETs formation. Therefore, I am examining NETs formation in neutrophils isolated from whole blood of healthy donors and from PiZZ (Glu342Lys) AAT-deficient (AATD) patients, suffering from chronic obstructive pulmonary disease (COPD). When compared to "usual" COPD, patients with PiZZ AAT deficiency (AATD)-related COPD have more excessive neutrophil infiltration and higher activity of elastase in the lungs. Whether NETs formation is more extensive in PiZZ relative to PiMM individuals is unknown. Since 25 years, augmentation therapy with AAT is used as a treatment for AAT deficiency-related COPD. Whether this therapy influences NETosis remains unknown. Answer to this type of questions will provide new insight into the mechanisms of functional activities of AAT and will give more insights into mechanisms how the immune system copes with pathogens by forming NETs.

I begin to examine NETs formation in blood neutrophils isolated from AATD patients before and directly after therapy with AAT. To evaluate my results I am using immunofluorescence stainings as well as transmission electron microscopy approaches. Fluorescently labeled antibodies from ImmunoTools, would help me to detect a variety of antigens in the NETs and consequently will help to reveal the role of AAT in this immunologcial process. Noticeably, not only enzymes (like elastase, myeloperoxidase or cathepsin G) directly involved into NETs, but also other molecules like histones H1, H2A, H2B, H3, and H4; pro/anti-inflammatory cytokines and chemokines (e.g. IL-6, IL-8, TNFalpha), and various proteins (e.g. calprotectin).

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Furthermore, it is still not yet clear if under some circumstances NETs release might be independent of cell death, bringing the Annexin V staining in the focus.

Revealing the effects of AAT on NETs formation is of clinical relevance, as our preliminary results suggest that AAT changes the shape of NETs, making them shorter and less adherent. Although highly speculative, AAT seems to help to assure the time-wise removal of NETs, which is a critical step in preventing the development of autoimmune diseases.

ImmunoTools *IT-Box-139.3* for **Eileen Frenzel** 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 CD45RO FITC, CD16 PE, IL-6 PE

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