# ImmunoTools *special* Award 2015

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## Pathological activation and survival of neutrophils

Neutrophils, also known as polymorphonuclear granulocytes (PMN), are the most common circulating leukocytes in human. They are the first line of defense against pathogens such as bacteria and fungi. To fight and kill these invaders, neutrophils contain a wide arsenal of cytotoxic weapons, including secreted proteases and bactericidal molecules (1). They also are able to engulf pathogens, and can produce high level of highly reactive oxygen species (ROS) (2) (1). Neutrophil can also produce extracellular traps, or NETs (3). NETs consist of neutrophil anti-bacterial proteins covered DNA that trap bacteria and prevent their dissemination in the organism (4). Moreover, recent progress has shown that neutrophil derived cytokines such as tumor necrosis factor alpha (TNF$\alpha$), interleukin 1 beta (IL-1$\beta$), IL-8, macrophage inflammatory protein (MIP-1) and vascular endothelial growth factor (VEGF) play important role during inflammation, and might be therapeutic targets of interest (5).

Neutrophils are also characterized by their relative short half-life. The differentiation of neutrophils, called granulopoiesis, takes place in the bone marrow. Once out of the bone marrow, the fully differentiated neutrophils will survive for 8 to 24 hours in the peripheral blood and up to 2 days in tissues (6). They eventually undergo apoptosis and are cleared by professional and non-professional phagocytes (7, 8). Resulting from this short life, an estimate of $1x10^9$ PMN per kg of body weight are renewed daily in humans (9). The mechanism of the spontaneous death of neutrophils is complex, involving extracellular signals and intracellular signaling pathways (7, 10). It is believed that the neutrophils quick and spontaneous death benefits the host by preventing the release of their highly cytotoxic and inflammatory

contents, as well as limiting the chance of intracellular parasites spreading. It is therefore one of the major regulators of the inflammatory responses.

While neutrophil deficiency leads to susceptibility to deadly infection, neutrophilia and hyper activity of neutrophils, on the other hand, is also detrimental. The high level of cytotoxic molecules secreted during sepsis by a large number of neutrophils can cause tissue damages and therefore worsen the inflammation (11). Accumulation of neutrophils in anti-neutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis (AASV) tissues may also occur as the result of a defective apoptosis (12). Inflammatory diseases like chronic obstructive pulmonary disease (COPD) (13), rheumatoid arthritis (RA) (14) or vascular inflammation (15) are the results of the failure of neutrophils to apoptose or the accumulation of activated neutrophils in tissues. It is known that unregulated neutrophil protease activities are linked to inflammatory lung diseases (16). Increased markers of neutrophil activation are also associated with the severity of some diseases. Therefore, molecules able to modulate neutrophil activities open the door to potential therapeutic applications.

The aim of our project is to examine the ability of organic compounds to modulate human neutrophil activation, functions and survival *in vitro*.

Therefore, ImmunoTools reagents will be very beneficial for the project in various aspects. First, for studying the neutrophil activation, we will evaluate the expression of various cell surface markers involved in neutrophil adhesion, an important step in activation. Panels of fluorescent-conjugated antibodies and flow cytometry will be useful for this part of the work. Moreover, as elongated life span reflects stimulated neutrophils, we plan to investigate neutrophils death over time after stimulation in different conditions by staining the cells with Annexin V. Finally, pro-inflammatory cytokine production by neutrophils can also be measured by ELISA. All in all, the combination of reagents available at ImmunoTools will allow us to explore the efficacy of the compounds of interest in the modulation of neutrophil activities, which might lead to the development of therapeutic agents of inflammatory diseases in the future.

## Bibliograpy


1. Segal AW. How neutrophils kill microbes. Annu Rev Immunol. 2005;23:197-223. PubMed PMID: 15771570. Pubmed Central PMCID: 2092448. Epub 2005/03/18. eng.
2. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. Annu Rev Immunol. 2012;30:459-89. PubMed PMID: 22224774. Epub 2012/01/10. eng.
3. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004 Mar 5;303(5663):1532-5. PubMed PMID: 15001782. Epub 2004/03/06. eng.

4. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular Neutrophil Extracellular Traps Capture Bacteria from the Bloodstream during Sepsis. Cell host & microbe. 2012 Sep 13;12(3):324-33. PubMed PMID: 22980329. Epub 2012/09/18. eng.
5. Kasama T, Miwa Y, Isozaki T, Odai T, Adachi M, Kunkel SL. Neutrophil-derived cytokines: potential therapeutic targets in inflammation. *Current drug targets Inflammation and allergy* 2005, **4**(3)**:** 273-279.
6. Edwards SW. Biochemistry and physiology of the neutrophil. Cambridge [England] ; New York: Cambridge University Press; 1994. xviii, 299 p. p.
7. Luo HR, Loison F. Constitutive neutrophil apoptosis: mechanisms and regulation. Am J Hematol. 2008 Apr;83(4):288-95. PubMed PMID: 17924549.
8. Mondal S, Ghosh-Roy S, Loison F, Li Y, Jia Y, Harris C, et al. PTEN Negatively Regulates Engulfment of Apoptotic Cells by Modulating Activation of Rac GTPase. The Journal of Immunology. 2011 December 1, 2011;187(11):5783-94.
9. Athens JW, Haab OP, Raab SO, Mauer AM, Ashenbrucker H, Cartwright GE, et al. Leukokinetic studies. IV. The total blood, circulating and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. J Clin Invest. 1961 Jun;40:989-95. PubMed PMID: 13684958.
10. Xu Y, Loison F, Luo HR. Neutrophil spontaneous death is mediated by down-regulation of autocrine signaling through GPCR, PI3Kgamma, ROS, and actin. Proc Natl Acad Sci U S A. 2010 Feb 16;107(7):2950-5. PubMed PMID: 20133633.
11. Hasan Z, Palani K, Rahman M, Thorlacius H. Targeting CD44 expressed on neutrophils inhibits lung damage in abdominal sepsis. Shock. 2011 Jun;35(6):567-72. PubMed PMID: 21330943. Epub 2011/02/19. eng.
12. Abdgawad M, Pettersson A, Gunnarsson L, Bengtsson AA, Geborek P, Nilsson L, et al. Decreased neutrophil apoptosis in quiescent ANCA-associated systemic vasculitis. PLoS One. 2012;7(3):e32439. PubMed PMID: 22403660. Pubmed Central PMCID: 3293802. Epub 2012/03/10. eng.
13. Snelgrove RJ, Jackson PL, Hardison MT, Noerager BD, Kinloch A, Gaggar A, et al. A critical role for LTA4H in limiting chronic pulmonary neutrophilic inflammation. Science. 2010 Oct 1;330(6000):90-4. PubMed PMID: 20813919. Pubmed Central PMCID: 3072752. Epub 2010/09/04. eng.
14. Chen M, Lam BK, Kanaoka Y, Nigrovic PA, Audoly LP, Austen KF, et al. Neutrophil-derived leukotriene B4 is required for inflammatory arthritis. J Exp Med. 2006 Apr 17;203(4):837-42. PubMed PMID: 16567388. Pubmed Central PMCID: 2118292. Epub 2006/03/29. eng.
15. Phillipson M, Kubes P. The neutrophil in vascular inflammation. Nat Med. 2011;17(11):1381-90. PubMed PMID: 22064428. Epub 2011/11/09. eng.
16. Zani ML, Baranger K, Guyot N, Dallet-Choisy S, Moreau T. Protease inhibitors derived from elafin and SLPI and engineered to have enhanced specificity towards neutrophil serine proteases. Protein Sci. 2009 Mar;18(3):579-94. PubMed PMID: 19241385.