Neutrophil phenotype orchestrates heart healing or failure post myocardial infarction

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Background and aim. Acute myocardial infarction (MI) is the leading cause of mortality and morbidity in Europe**.** Neutrophils are the initial cell responders that massively infiltrate the infarct area in the first few hours following the onset of ischemia. Although traditionally considered as detrimental in the setting of an acute MI, recent evidences demonstrated that neutrophils could also play a significant role in cardiac repair, and reveal the existence of heterogeneity and versatility of neutrophils. The data obtained in the last decade showed that different neutrophils subpopulation exhibit transcriptional changes. Two subpopulations of neutrophils were defined post-MI, the N1 pro-inflammatory and N2-anti-inflammatory neutrophils. We **hypothesized** that N1 and N2 neutrophils may have specific transcriptomic profiles that can dictate the disease evolution. To test this hypothesis, we **aimed** at performing a complex analysis to identify specific markers and functions of these neutrophil subtypes.

Results. Our RNAseq results showed that N1 neutrophils exhibit a distinct inflammatory phenotype up-regulating a large number of inflammatory cytokines and chemokines CCL3, CCL4, CCL5, IL-12a, TNF-α and IL-12α that are involved in leukocyte recruitment and in the inflammatory process. Conversely, N2-neutrophils expressed molecule associated with anti-inflammatory phenotype IL-4, CD206, Ym1 and Arg1 while displaying a reduced expression of inflammatory molecules such us IL-1β, TNF-α and CXCL16. Functional analysis revealed that unlike N2-neutrophils, N1 neutrophils showed higher oxidative burst and ROS levels, increased activity of MPO and MMP-9 and an enhanced chemotactic activity. To reveal whether N1 and N2 neutrophils affect macrophages (MAC) polarization, the latter were exposed to the secretome collected from N1 or N2-neutrophils. The results showed that MAC exposed to secretome from N1 cells exhibited increased expression of IL-1β and TNF-α, and MAC exposed to secretome collected from N2 cells enhanced the expression of efferocytosis and anti-inflammatory molecules, Mertk, MGF-E8 and TGF-β. The data demonstrated that factors released by N1 or N2 neutrophils induced selectively either M1 or M2 Mac polarization.

Conclusion. Our data define novel specific inflammatory markers and functional features of N1 and N2-neutrophils. In addition, the results highlight the existence and the role of the cross-talk between neutrophils and macrophages on specific MAC polarization towards the pro-, or anti- inflammatory cells and the subsequent fate of MI. Together, the data suggest that neutrophils show a great level of plasticity and develop distinct phenotypes and functionality in response to pathological (e.g. inflammation) conditions, that may orchestrate the disease progression by inducing pro or anti-inflammatory molecules and the ensuing pro- and anti-inflammatory macrophages.

Acknowledgments. This work was supported by a grant from the Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0172, within PNCDI III and the Romanian Academy.