METHODS TO DETECT STRESS RESPONSE IN BLOOD PHAGOCYTES

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Key words: myeloid blood cells, phagocytosis, stress, autoimmune disorders

The ability of myeloid blood cells, particularly neutrophils and monocyte, to engulf and digest objects constitute the important stress-defense mechanism of the body. These object are both pathogens – bacteria or viruses, virus-infected cells, dying cells and debris. Besides, blood samples can be readily obtained and used to evaluate individual response to stress of particular person and under specific treatments. Both engulfment (phagocytosis) and subsequent digestion of harmful material is needed to accommodate the stress action. The latter process is usually accompanied by activation of ROS-producing machinery aimed to kill potentially harmful pathogens (bacteria).

It is known that inappropriate clearance of apoptotic cells is a primary cause of autoimmune disorders. This defective clearance of apoptotic cells causes secondary necrosis with a release of intracellular content and inflammatory mediators. Rapid cold stress decrease phagocytic activity. Reduced phagocytosis observe in patients with recurrent bacterial skin, wound infections from burns, AIDS etc. Also reduced phagocytic activity of neutrophils was found as a negative predictor for survival of patients with sepsis. At the same time, failure of ROS-producing machinery results in efficient phagocytosis without destruction of pathogens, in organism level resulting in Chronic granulomatous disease (CGD) and related pathologies. ROS-production is critical component of neutrophil activation, followed by production of neutrophil extracellular traps (NETs).

Currently available tests for phagocytosis and activation (often referred as "phagoburst" to reflect respiratory burst of phagocytes) of blood phagocytes usually have drawbacks due to different activity of monocytes and neutrophils to be tested in one tube. The aim of current work was to develop the method for evaluation of the phagocytosis of bacteria by whole blood cells (neutrophils and monocytes) and to access their ability to produce ROS species.

The *Escherichia coli* bacteria that used in the test was opsonized with immunoglobulin and complement of pooled sera. And it showed very good results with neutrophils activation but not with monocytes. Thus, different bacteria strains were tested for their capacity to activate neutrophils and monocytes. It was found that adherent-invasive *E. coli* (AIEC) bind and penetrate into human monocytes-derived macrophages better compared to non-pathogenic bacteria (strain K12) and strain UTI89 causing urinary tract infection. Specific labeling of monocytes allowed to discriminate monocyte response in hyper- and hypoactive conditions. The utilization of the developed approach to test ROS production in neutrophils and monocytes allowed us to test the potential drug candidates aimed to enhance diminished phagoburst activity of blood phagocytes.

This work allowed to develop and optimize an effective method for detecting the phagocytic ability of whole blood monocytes and neutrophils.

Acknowledgement: This work was carried out with financial support from the Ministry of Healthcare in Ukraine (project 0116U000759), EU Horizon2020 project "Pathogens and Graphen" PANG/690836.