Proteomic Comparative Analysis Between Quiescent And fMLP Stimulated Neutrophils

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In the initial phase of combating invading organisms, stimulation and activation of neutrophils is mandatory. These cells are capable of being activated by several substances, including N-formyl-metionil-leucil-phenylalanine (fMLP) a peptide originated from the degradation of bacterial or mitochondrial proteins. In this study, neutrophils from three healthy donors were separated using Percoll gradients, activated with fMLP(100nmol/L), and assayed by flow cytometry confirming activation by release of ROS using DHR`1,2,3. Cell lysis was performed in urea/thiourea/detergent buffer, followed by determination of protein concentration by the Bradford method. For 2D-PAGE, triplicate samples of each individual from each group were applied in 4-7 IPG gels and then submitted to SDS-PAGE. Silver stained gels were scanned and the images were processed in the Image Master software for detection and matching of the gels. In the gel from quiescent neutrophils, 1468 spots were detected while the fMLP activated cells revealed 1490 spots. Comparison between the master gels shows 545 unique spots to quiescent neutrophils and 532 spots unique to activated cells. After quantitative comparison of the gels in quiescent conditions and activated, the variation in the percentage of the spots volume revealed 95 spots upregulated and 102 downregulated after fMLP activation. Some spots were identified by PMF and MS/MS, among them 8 with differential expression, being 2 with decreased expression and 6 increased. The identification of such proteins is a starting point for the selection of molecular markers to be used either as diagnostic of bacterial diseases or as therapeutic to inflammatory problems such as the systemic inflammatory response, related to the activation of neutrophils.

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