Proteomic Analysis of HDL Function in Macrophages during Inflammatory Disease

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Monocytes are immune cells present on blood circulation and represent 10% of all leucocytes in the whole body. During inflammatory conditions, monocytes are recruited and differentiated to macrophages, responsible to adhere to the tissues and to secrete cytokines, chemokines and reactive species, as nitric oxide, to eradicate pathogens. Recent studies evaluating gene expression, showed that high-density lipoprotein (HDL) has anti-inflammatory properties, reducing the levels of toll-like receptor 4 (TLR4) and suppressing secretion of IFN-a and IFN-b, inhibiting type I interferon response. However, there is no proteomic study corroborating this affirmation. For monocytes isolation, 50mL of heparinized whole blood was mixed with PBS and Dextran to separate white cells from other blood components. The mixture of leucocytes was centrifuged with Hystopaque to obtain the Peripheral Blood Mononuclear Cells (PBMC) interface, which was collected to isolate monocytes by negative selection method using Dynabeads. Monocytes were differentiated to macrophages by phorbol 12-myristate 13-acetate (PMA) for two days. Then, macrophages were incubated with HDL particles for 2h, followed by the addition of lipopolysaccharide (LPS) for 3h, for the HDL+LPS group. As controls groups, there were non-treated cells, HDL-only and LPS-only group. Next, the secretome was collected and HDL particle was isolated from the macrophage secretome by ultracentrifugation, that was also used to enrich for macrophage membrane proteins. Samples were digested and analyzed on a high-resolution nano LC-MS/MS. After digestion, aliquots of cell lysate were fractionated by UPLC to create a library. The investigation of the HDL effects started by the analysis of the secretome, considering that macrophages typically secrete characteristic proteins during an inflammatory immune response. Subgroup analysis showed that LPS only secretes proteins with biological process (BP) related to “antigen processing and presentation”, “response to bacterium”, “innate immune system”, “trafficking and processing endosomal TRL” and “MHC class I peptide loading complex”. Analyzing the classification of the secreted proteins by gene ontology, the classification “external side of plasma membrane” indicated an exclusive protein for the LPS only-group, the SLAM family member 5 (CD84) protein, which is known to enhance MAPK phosphorylation induced by LPS and activate NF-kb, responsible to modulate cytokines secretion during LPS inflammation. On the other hand, HDL+LPS group attenuated these processes, enriching only for common metabolic processes, which indicates a possible anti-inflammatory function for the HDL particle. Also, other interesting BPs “cellular response to type II interferon” and “cellular response to cytokines stimulus” were enriched when analyzing the most abundant proteins of the subgroups HDL only and HDL+LPS together. This can lead to the hypothesis that the TRAM/TRIF pathway is suppressed by HDL, so the production of type I interferons is reduced, enhancing the production of IFN-g, that stimulates the macrophage to eliminate the antigen by phagocytosis, process enriched by KEGG analysis of HDL treated groups. The results obtained indicate that the HDL particles may regulate the immune response of macrophages during inflammatory conditions initiated by TLR4 signaling activated by the LPS stimulus. More data is underway to comprehend the mechanism of action of the HDL in the immune system.

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