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Effect of hyperglycemia on the activation and epigenetic programming of primary human macrophages

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Hyperglycemia is hallmark of diabetes that is related to the development of diabetic vascular complications. Macrophages are key innate immune regulators of inflammation that undergo two major vectors of functional polarisation: classically (M1) and alternatively (M2) activated macrophages. Both M1 and M2 types of macrophages play a role in diabetes. M1 are involved in the establishment and progression of insulin resistance and inflammatory processes leading to vascular complications, whereas M2 can have protective effects in diabetes by reducing inflammation, obesity and insulin resistance. However, the effect of hyperglycemia on differentiation and functional programming of macrophages is poorly understood. In order to analyse detrimental effects of high glucose on the differentiation and activation of monocytes and macrophages we established new model system based on primary human monocyte-derived macrophages cultured in serum-free conditions in the presence of 5mM and 25mM glucose. Effects of high glucose were examined in control (M0), classically (M1) and alternatively (M2) activated macrophages. Using RT-PCR and ELISA the expression and release of TNF-alpha and IL-1beta (M1 cytokines) and IL-1Ra and CCL18 (M2 cytokines) were quantified. Hyperglycemia stimulated production of TNF-alpha, IL-1beta and IL-1Ra during macrophage differentiation. The effect of hyperglycemia on TNF-alpha was acute, while the stimulating effect on the production IL-1beta and IL-1Ra was continuous during monocytes to macrophage differentiation. Production of CCL18 was suppressed in M2 macrophages by hyperglycemia. Altogether analysis of the cytokine release indicated that hyperglycemia itself, independently on other metabolic factors, can induce mixed M1/M2 cytokine secretion profile that can support the progression of diabetes and vascular complications. In order to identify differentially expressed genes in M0, M1 and M2 macrophages differentiated in normal and high glucose conditions, Affymetrix DNA microarray was used. We found that hyperglycemia induced differential expression of 1171 genes in M0, of 1573 genes in M1 and of 16 genes in M2. The major affected groups of differentially expressed genes were: chemokines, cytokines, chemokine receptors, glycoproteins family, RNase A family, S100 calcium binding protein family, solute carrier family, transmembrane protein family and zinc finger family. Hyperglycemia had very strong inducing effect on the expression of CCR2, major receptor for macrophage chemotactic factor CCL2 that mediates recruitment of macrophages in chronic inflammation. The ability of hyperglycemia to enhance trans-migratory activity of macrophages was analyzed in a trans-well system. Significantly higher amounts of M0 (7.6 times increase) and M1 (11.2 times increase) transmigrated towards CCL2 (100 ng/ml) in the hyperglycemic conditions. In consistence with the strong induction of CCR2 expression, hyperglycemia also induced migration of M1 macrophages towards CCL2 even when it was used in very low concentrations (up to 1.56 ng/ml). Histone code was demonstrated to be essential mechanism that control macrophage differentiation in inflammatory conditions. However the role of histone code in the hyperglycemia-mediated programming of human macrophages remained unknown. Chromatin immunoprecipitation assay (ChIP) was applied to examine the presence of histone marks on the promoters of these genes. Three active histone modifications (acetylation of histone H3(aceH3), H3K4me3 and H3K4me1) and two repressive histone modifications (H3K9me3 and H3K27me3) at the promoters of CCR2 and IL-1beta genes were analyzed in the primary human macrophages cultured in normal and hyperglycemic condition. It was demonstrated that hyperglycemia caused a statistically significant increase in the level of histone activating marks H3K4me1 and H3K4me3 at the CCR2 promoter and IL-1beta promoters. Hyperglycemia did not affect repressing histone marks on the CCR2 and IL-1beta gene promoters. Analysis of macrophages isolated from individual donors demonstrated that levels of activating histone marks on CCR2 and IL-

1beta promoters corresponded to the levels of up regulation of their gene expression. The cooperation of H3K4me1, H3K4me3 and AcetylH3 was required for efficient stimulation of CCR2 gene expression, while cooperation of H3K4me1 and H3K4me3 was critical for stimulation of IL-1beta gene expression in hyperglycaemic conditions. Tri-methylation of H3K4 is mediated by MLL group of enzymes, and our study for the first time suggests that MLL enzymes can be involved in the hyperglycemia-mediated epigenetic programming of macrophages. In summary, we found that hyperglycemia induced expression of CCR2 and IL-1beta on primary human macrophages is linked to the epigenetic modifications of CCR2 and IL-1beta promoter by activating histone code. Elevated levels of CCR2 resulted in high sensitivity of macrophages to the chemotactic ligand CCL2. Our data suggest that hyperglycemia can be a primary factor that induces attraction of pro-inflammatory macrophages into the sites of low grade inflammation that can affect progression of vascular complications at very early stages.