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The impact of hyperglycemia on CD163-mediated scavenging of hemoglobin-haptoglobin complexes in human primary monocyte-derived macrophages

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Hyperglycemia is a hallmark of diabetes and can induce inflammatory programming of monocytes and macrophages. Scavenging function of monocytes and macrophages is essential for the clearance of both exogenous components and unwanted self-products and the control of inflammatory reactions. Scavenger receptor CD163 internalizes hemoglobin-haptoglobin (Hb-Hp) complexes to initiate their degradation. The genetic variants haptoglobin1-1 (Hp1-1) and haptoglobin2-2 (Hp2-2), considerably differing in their molecular structure, form complexes with free hemoglobin in situations of intravascular hemolysis which especially occurs in inflammatory conditions. Inflammation is an essential pathological process leading to the development of micro- and macrovascular diabetic complications. Clinical studies have found a correlation between an impaired scavenging process of Hb-Hp complexes via CD163, particularly in combination with the presence of the genetic variant Hp2-2, and vascular complications of diabetes mellitus. Our aim was to identify the effect of hyperglycemia on CD163 expression and function in human primary macrophages.

We used the model system based on human primary monocyte-derived M(IFNγ) and M(IL-4) macrophages that was established in our laboratory. In this system, M(IFNγ) and M(IL-4) macrophages as well as non-stimulated M0 macrophages were differentiated out of human monocytes in normal (5mM) and high (25mM) glucose conditions in the presence of IFNγ and IL-4 or without further cytokine stimulation. The effects of hyperglycemia on CD163 gene and surface expression were quantified by RT-qPCR and flow cytometry. The function of CD163 was analysed by flow cytometry using fluorescently labeled Hb-Hp1-1 and Hb-Hp2-2 complexes, the uptake being visualized by confocal microscopy. The effect of hyperglycemia on the release of soluble CD163 and of read-out inflammatory cytokines TNFα, IL-1β, IL-6, IL-8 and IL-1RA was detected by ELISA.

CD163 gene expression was decreased 5.53 times in M(IFNγ) and 4.76 times in M(IL-4) compared to non-stimulated M0 macrophages. Hyperglycemia elicited an additional suppression of CD163 gene expression only in M(IFNγ) (1.99 times). CD163 surface expression was downregulated in a statistically significant way by hyperglycemia in M(IFNγ) (1.43 times); but not in M(IL-4). In normoglycemic conditions, the shedding of surface CD163 proved to be most effective in pro-inflammatory conditions, whereas in hyperglycemia, the cleavage of CD163 was most pronounced in M(IL-4). Flow cytometry and confocal microscopy demonstrated that hyperglycemia did not impair Hb-Hp complex uptake and delivery to endosomal pathway, neither in inflammatory nor in anti-inflammatory macrophages. Hb-Hp1-1 complex uptake was the strongest stimulus for M(IFNγ) for the acute (6h) release of TNFα, IL-1β, IL-6, IL-8 and IL-1RA, with IL-6 secretion being enhanced the most (3.03 times). However, 24h after Hb-Hp1-1 complex uptake, cytokine release decreased in normoglycemia, the strongest effect being detected for IL-6 (5.78 times). Hyperglycemia interfered with Hb-Hp1-1 mediated suppression (only 3.05 times suppression of IL-6 release). Contrarily, Hb-Hp2-2 complex uptake did not affect cytokine release in a significant way after 6h, but increased the secretion of all read-out cytokines after 24h, IL-6 release being stimulated the most (3.06 times). Overall, the strongest enhancing effect of hyperglycemia was detected for IL-1β release 24h after M(IFNγ) were stimulated with Hb-Hp1-1 complexes (6.75 times).

Our data suggest that the inflammatory response of M(IFNγ) after Hb-Hp1-1 and Hb-Hp2-2 complex uptake in hyperglycemic conditions can enhance low-grade inflammation in patients with diabetes mellitus and promote their susceptibility to vascular complications by a dysfunctional control of tissue damage.