Detection of cytokines and chemokines in supernatants and blood serum of patients with diabetes mellitus type 2 by multiplex analysis

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Human cytokines and chemokines constitute group of molecules responsible for recruitment of immune cells to the sites of inflammation and for organization of spatial-temporal relationships between cells. Diabetes mellitus type 2 (DM2) represents one of the major cardiovascular risk factors and is known to be associated with development of inflammation. The aim of the present study was to evaluate spontaneous and lipopolysaccharide (LPS)-stimulated production of major cytokines and chemokines by peripheral blood mononuclear cells (PBMCs) in patients with DM2 and to compare it with the level of corresponding molecules in blood serum using xMAP technology.

Methods: In total 14 patients with arterial hypertension (AH) were recruited in the study (6 men and 8 women; mean age 57.7±1.8 y.o.; mean level of glycated hemoglobin 7.8±0.5 %; obtained values of office blood pressure<140/85 mmHg). AH was associated with DM2 in 7 patients, while 7 patients had AH without DM2. Blood samples were obtained after overnight fasting. PBMC were prepared and were either left intact or stimulated with 10 ug/ml LPS for 24 hours. Concentrations of interleukin (IL)-1 beta (1β), IL-1β receptor antagonist, tumor necrosis factor-alpha (TNF-α), IL-12p40, IL-12p70, interferon gamma-induced protein 10 (IP-10), macrophage-derived chemokine (MDC, CCL2) in samples of supernatants and blood serum were detected on Multiplex Instrument FLEXMAP 3D Luminex Corporation using MILLIPLEX map Human Cytokine/Chemokine Panel I and MILLIPLEX Analyst 5.1 software (MERCK, Millipore, Milliplex; USA).

Results: We revealed elevation of macrophage-derived chemokine (MDC, CCL2) in LPS-stimulated supernatants and serum of patients with DM2 compared to the patients without DM2 (1124.3±429.2 pg/ml vs. 686.1±292.4 pg/ml; p=0.037 in supernatants and 922.5±187.1 pg/ml vs. 641.1±175.8 pg/ml; p=0.022). At the same time, concentration of one of the most well studied cytokines, tumor necrosis factor alpha (TNF-α) was increased only in serum of patients with DM2 compared to the patients without DM2, but not in supernatants (19.0±2.4 pg/ml vs. 13.4±3.9 pg/ml; p=0.005). Concentrations of other cytokines and chemokines did not differ between patients with DM2 and patients without DM2. This may be due to such limitation of our study as a rather small number of recruited patients.

Conclusions: Patients with DM2 are characterized by elevation of both LPS-induced secretion of MDC and MDC level in blood. According to the recent studies of other authors MDC plays an important role in recruitment of immunosuppressive T-regulatory cells. Our data prompts that macrophage-derived chemokine may be used as a possible biomarker of PBMC activation in patients with association of AH and DM2, and activation of its production may have compensatory function. We regard this preliminary data obtained after our first experience of using xMAP technology for biomarkers evaluation as an important perspective for our future studies of immune dysregulation in patients with association of AH and DM2.