Membrane expression of Toll-like receptors (TLR) and intracellular cytokine synthesis in patients with chronic kidney disease and diabetic nephropathy

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Inflammation plays an important role in the pathogenesis of chronic kidney disease (CKD). Toll-like receptors (TLRs) are a family of pattern recognition receptors that participate in the regulation of immune function and inflammatory responses of certain pathological conditions. In this study we investigated the membrane expression of TLR2 and TLR4 on monocytes as well as the intracellular cytokine synthesis in CKD patients with and without diabetic nephropathy. We also measured the serum cytokine levels. In this pilot, cross-sectional study, 56 CKD patients of stages 1-4 were included with a mean eGFR – MDRD of 36.7±22 ml/min/1.73 m². Patients were divided in two groups, pending on having or not diabetic nephropathy. Group 1, included 37 CKD patients, (25 men, 12 women, mean age 66.4±12.5 years) not having diabetes mellitus. Group 2 included 19 CKD patients (14 men, 5 women, mean age 69.3±9.0 years) with diabetic nephropathy. Both groups were compared with 21 age matched controls, (control group). Patients receiving statins, or having cancer or autoimmune disease or have been hospitalized recently for infectious diseases, were excluded from the study. Membrane expression of TLR2 and TLR4 was determined by staining with anti-CD282-PE and anti-CD284-PE antibody respectively, and analysis of mean fluorescence intensity (MFI) was performed by flow cytometry. In addition, we studied the intracellular cytokine (IL-6 and IL-1β) synthesis in 10 patients of each group, before and after stimulation with lipopolysaccharide (LPS), labeled with anti-CD14 antibody. In all patients and healthy controls, we measured the serum cytokine levels of IL-1β and IL-6 and of 25 additional cytokines through method of multiple mapping (xMAP).

Patients of group 1 exhibited increased membrane expression only of TLR2 in monocytes compared with the control group (MFI: 136±36 versus 116±21, P<0.02). Patients of group 2 presented increased membrane expression of both TLR2 and TLR4 compared with the control group (MFI: 148±40 and 61±28 versus 116±21 and 39±10, P<0.003 and P<0.001, respectively) and increased expression of TLR4 compared with group 1 (MFI: 61±28 versus 46±19, P<0.02). Both the stimulated and unstimulated intracellular monocyte cytokine levels were reduced in the studied groups compared to control and there were no statistically significant differences between the two CKD groups. No differences of IL-1β levels were detected between group 1 and group 2 and control group. Both group 1 and 2, presented increased levels of IL-6 compared with control group.

CKD patients and patients with diabetic nephropathy are characterized by increased expression of TLRs, on monocytes, that may contribute to their increased inflammatory state. The reduced levels of intracellular cytokines in both groups indicate impaired signal transduction, possibly due to CKD.