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The role of tumour necrosis factor (TNF) and Interleukin-6 (IL-6) in the immunopathogenesis of experimental autoimmune tubulointerstitial nephritis (TIN)

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The quantity of the proinflammatory cytokines TNF and IL-6 is increased in the sera of BALB/c mice with TIN. It shows that they play a pathogenic role as mediators of the renal inflammation.

Key words: Cytokines, Experimental Interstitial Nephritis.

Introduction

Recently the cytokines, which take part in the tissue destruction in the renal diseases are an object of the intensive studies. Most of the investigations concern the glomerular kidney diseases [1]. There are not data in the literature about the role of cytokines as mediators of autoimmune processes in the renal tubulointerstitial injuries. Recently it has been established that IL-1α is a mediator for kidney inflammation in experimental autoimmune TIN [10]. It is evident that IL-1 has a synergic action with TNF [2, 13]. There are two types of TNF — α and β, which have a similar biological action in the inflammation and the antitumour activity. But the cells synthesizing them are different. The activated monocytes/macrophages are the main resource of TNFα. TNFβ is produced from T1 helper lymphocytes. The biological effects of TNF are connected with the induction of IL-1 secretion, and with the increase of the expression of Major Histocompatibility Complex (MHC) class I molecules and adhesion molecules. TNF has a cytotoxic effect on the michene cells [3]. Both TNF and IL-1 are involved in the nonspecific immune

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response. They are important inductors of acute-phases proteins. The hightened production of TNF play an important role in pathological processes particularly in the cachexion and autoimmune diseases [6].

IL-6 is a proinflammatory cytokine which is known as a B cell stimulatory factor needed for the differentiation of B cells and antibody production. It is synthesized from different immune and nonimmune cells as monocytes, macrophages, T and B lymphocytes, fibroblasts, endothelial, mesangial cells and so on. Using IL-6 transgenic mice it is shown that IL-6 participates in the pathogenesis of glomerulonephritis [14]. The levels of IL-6 in the urine correspond to the degree of the histological kidney damages which give a possibility to prognose the progress of the diseases [4, 5].

That all motivate us to investigate TNF and IL-6 production in the experimental autoimmune TIN, a prototype of many renal diseases in man.

Material and Methods

The investigation is done with 30 BALB/c inbred mice. Tubular basement membrane (TBM) antigen is prepared after the method of Ulich et al. [15]. Briefly, bovine renal cortical tubules are isolated by passing the dissected renal cortex through metal sieves. TBM is prepared by ultrasonic disruption of isolated tubules. Each animal received i.d. 1μg TBM antigen emulsified in CFA. The mice are sacrificed at the 2nd and the 4th month after the immunization. Material for routine histological examination is taken from the kidneys, fixed in 10% formalin and prepared paraffin section stained with hematoxilin and eosin.

Cytokine activity immunoassay. Sera from different experimental groups of animals are used.

TNF bioactivity assay. Murine L-929 fibroblasts are used as a target cell line [8]. THF cell activity is measured (U ml^{-1}) in a standard colorimetric microtoxicity assay. The probes are analized with ELISA reader at an absorbance of 595 nm.

IL-6 assay. IL-6 activity is measured by photometric enzime immunoassay kit (Boehringer Mannheim). The plates are measured at 450 nm using ELISA reader. The concentration of IL-6 in the samples is detected using the standard curve in pg. ml^{-1}.

Statistical analysis. Statistical analysis is performed using Student-Fischer T-test for significant differences.

Results and Discussion

The results demonstrate an increased TNF and IL-6 bioactivity in all samples from TIN animals in comparison with the healthy controls (Fig. 1— *a, b*).

Our results show that TNF and IL-6 are mediators of the inflammation in the experimental autoimmune TIN. The increase of TNF in the sera of BALB/c mice with TIN could be due to its secretion from activated monocytes. It is possible the secretion to be from T lymphocytes, which are hightened in TIN [10].

The human and mouse IL-6 are egually active on the mouse cells [7]. They have 65% homology in the nucleotide sequences and 42% — in aminoacid sequences. It means that human monoclonal antibodies against IL-6 are suitable for the determination of mouse IL-6 in the sera of the experimental animals. The increased quantity of IL-6 in TIN could be due to the activation as well as of T-helper cells as of B lymphocyte populations, which are enhanced in TIN [9]. As a B cell stimulatory factor IL-6 is important for the differentiation of B cells [11]. This leads to the rising

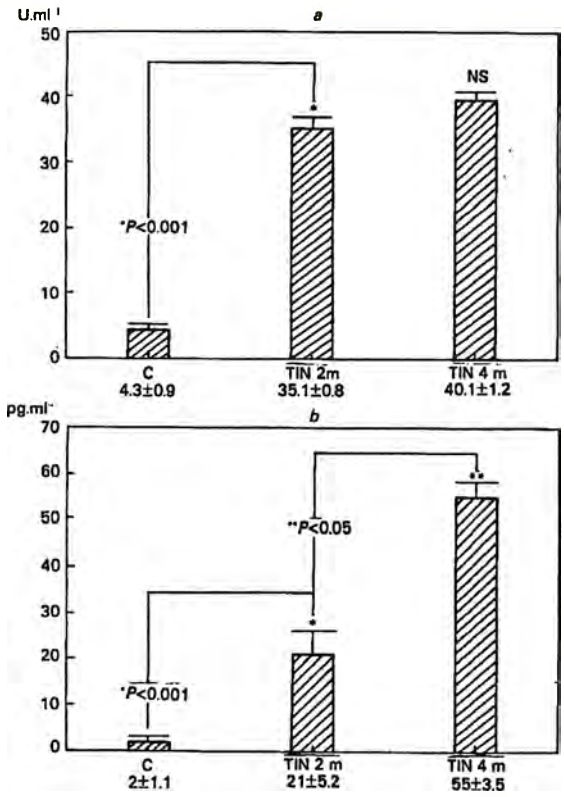

Fig. 1. Increased TNF activity (a) and IL -6 activity (b) in the sera of BALB/c mice with experimental autoimmune TIN at the 2nd and 4th month after the induction of the disease. Data are mean ± SD
C—Control, healthy animals non-immunized with TBM antigen; TIN2m—Animals at the 2nd month after immunization with TBM;TIN4m—Animals at the 4th month after immunization with TBM *P<0.001 vs C; NS vs TIN2m
(b) C—Control, healthy animals non-immunized with TBM antigen;TIN2m—Animals at the 2nd month after immunization with TBM; TIN4m—Animals at the 4th month after immunization with TBM *P<0.001 vs C, **P<0.005 vs TIN2m

of specific TBM antibodies which play a basic role in TIN [12]. Our results show that IL-6 is a pathogenic mediator of the inflammation in experimental autoimmune TIN.

The cytokines are not used in the therapy of kidney diseases because only some aspects of their expression and production in pathology are known. Our results can allow an inhibition of the effect of these cytokines which will be an object of our future work.

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