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ORIGINAL ARTICLE

T-lymphocyte-expressing inflammatory cytokines underlie persistence of proteinuria in children with idiopathic nephrotic syndrome☆

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KEYWORDS
Idiopathic nephrotic syndrome;
Cytokines;
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Abstract
Objective: There is evidence of an important role of immune system changes in the triggering and maintenance of idiopathic nephrotic syndrome (INS). The aim of this study was to investigate the expression of cytokines in lymphocyte populations of patients with INS in comparison to healthy individuals, according to proteinuria.
Methods: This cross-sectional study included 44 patients with INS and eight healthy children, matched for age and sex (controls). Patients were subdivided according to proteinuria: persistent proteinuria or partial remission (PP $\geq$ 300 mg/24 h, $n = 17$) and low proteinuria or complete remission (LP < 300 mg/24 h, $n = 27$). *Ex vivo* analysis of peripheral blood leukocytes by flow cytometry was performed using surface markers for T-lymphocytes, TCD4, TCD8, NK cells, NKT, and B-lymphocytes. Frequencies of intracellular cytokines were analyzed in these cells.
Results: The frequencies of B-lymphocytes, NK cells, and NKT cells were lower in INS than in controls, whereas INS patients had a higher frequency of $CD4^+TNF\text{-}\alpha^+$ cells than controls. Cytotoxic-T-lymphocytes expressing IFN-γ were lower in INS than in controls. Patients with PP

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showed higher frequencies of CD4-T-lymphocytes expressing IFN-γ and TNF-α than controls. CD8-lymphocytes expressing TNF-α were increased in PP group when compared with LP and controls, while $CD8^{+}IFN\text{-}\gamma^{+}$ cells were lower than in LP and in controls.
Conclusion: Regardless the level of proteinuria, INS patients had increased expression of TNF-α in CD4-lymphocytes and reduced expression of IFN-γ in CD8-lymphocytes. Persistence of proteinuria was associated with higher levels of inflammatory markers.

PALAVRAS-CHAVE
Síndrome nefrótica idiopática;
Citocinas;
Inflamação;
Proteinúria;
Resposta imune

Os linfócitos T que expressam citocinas inflamatórias são subjacentes à persistência de proteinúria em crianças com síndrome nefrótica idiopática

Resumo
Objetivo: Há comprovação do importante papel das alterações no sistema imunológico no desencadeamento e manutenção da síndrome nefrótica idiopática (SNI). O objetivo deste estudo foi investigar a expressão das citocinas em populações de linfócitos de pacientes com SNI em comparação a indivíduos saudáveis e de acordo com a proteinúria.
Métodos: Este estudo transversal incluiu 44 pacientes com SNI e oito crianças saudáveis, pareados por idade e sexo (controles). Os pacientes foram subdivididos de acordo com a proteinúria: proteinúria persistente ou remissão parcial ($PP \geq 300\,mg/24\,h$, $n = 17$) e proteinúria baixa ou remissão completa ($PB < 300\,mg/24\,h$, $n = 27$). A análise *ex vivo* de leucócitos no sangue periférico por citometria de fluxo foi feita utilizando marcadores de superfície para linfócitos T, TCD4, TCD8, células NK, linfócitos NKT e B. As frequências das citocinas intracelulares foram analisadas nessas células.
Resultados: A frequência dos linfócitos B, células NK e células NKT foi menor em pacientes com SNI do que nos controles, ao passo que os pacientes com SNI apresentaram maior frequência de células $CD4^{+}TNF\text{-}\alpha^{+}$ do que nos controles. Os linfócitos T citotóxicos que expressam IFN-γ foi menor nos pacientes com SNI do que nos controles. Os pacientes com PP mostraram maiores frequências de linfócitos T CD4 que expressam IFN-γ e TNF-α que os controles. Os linfócitos CD8 que expressam TNF-α apresentaram aumento no grupo com PP, em comparação aos com PB e os controles, apesar de as células CD8+IFN-γ+ serem mais baixas nos pacientes com PB e nos controles.
Conclusão: Com relação ao nível de proteinúria, os pacientes com SNI apresentaram aumento na expressão de TNF-α nos linfócitos CD4 e expressão reduzida de IFN-γ nos linfócitos CD8. A persistência da proteinúria foi associada a maiores níveis de marcadores inflamatórios.

Introduction

Nephrotic syndrome (NS) is a very common glomerulopathy in children, characterized by massive proteinuria, hypoalbuminemia, generalized edema, and hyperlipidemia. NS can be caused by primary renal lesion, referred as idiopathic nephrotic syndrome (INS), or be related with systemic illnesses.[1]

There is evidence of an important role of immune system changes in the triggering and maintenance of INS.[2–5] These changes include abnormal T-lymphocyte response in patients with INS,[2] a possible contribution of cytokines in INS physiopathology,[3] the possibility of a circulating permeability factor that has been related with the recurrence of the illness after renal transplant,[3] and the clinical improvement of patients after treatment with corticosteroids and immunosuppressive medications.[4] However, apart from the advances on INS research over the past decades, especially on the immunology field, its physiopathology remains to be fully addressed.[5]

Several immunological alterations have been detected in patients with INS.[5] There are reports of altered expression of cytokines/chemokines, including interleukin-2 (IL-2), IL-10, IL-4, and IL-8, as well as changes in $TCD8^{+}$ and in $TCD4^{+}$ cells of INS patients.[2,6–10] IL-2 and tumor necrosis factor alpha (TNF-α) are considered possible pathogenic factors underlying the mechanisms of renal lesion in INS.[11,12] For instance, the infusion of TNF-α in mice with NS caused a dose-dependent increase of proteinuria in parallel with impairment of clinical state.[13] Importantly, the increase of plasma levels of TNF-α in INS patients associated with increased plasma levels of IL-2, its soluble receptor (sIL-2R), and IFN-γ during NS relapses in steroid-sensitive patients suggests a pro-inflammatory or T-helper 1 (Th1) immune response pattern.[14] However, an imbalance between Th1 and Th2 immune response patterns has