The clinical course of steroid-sensitive childhood nephrotic syndrome is associated with a functional IL12B promoter polymorphism

Jan Müller-Berghaus1,2, Markus J. Kemper3, Bernd Hoppe4, Uwe Querfeld5, Dirk E. Mühler-Wiefel3, Grant Morahan6, Dirk Schadendorf1 and Klaus Tenbrock7

1Skin Cancer Unit, German Cancer Research Centre, Heidelberg, 2Paul-Ehrlich-Institut, Langen, 3Department of Paediatric Nephrology, University Children's Hospital, Hamburg, 4Department of Paediatric Nephrology, University Children's Hospital of Cologne, 5Department of Paediatric Nephrology, Charité University Hospital, Berlin, Germany, 6School of Medicine and Dentistry, University of Western Australia, Perth, Australia and 7Department of Paediatrics, University of Aachen, Germany

Abstract

**Background.** Steroid-sensitive nephrotic syndrome (NS) of childhood is the most common glomerular disease in children. The type and duration of response to corticosteroid therapy are used for clinical classification, and especially patients with steroid dependence often have a complicated course, requiring intensified immunosuppressive treatment. Its cause is still unknown although a cytokine-mediated course of disease has been implicated. Interleukin 12 (IL-12) is critical in determining the type of immune response. The ability of dendritic cells to secrete bioactive IL-12 is associated with a bi-allelic polymorphism within the promoter region of IL12B, the gene encoding the IL-12 p40 subunit. We hypothesized that this genotype may be involved in steroid-sensitive INS.

**Methods.** Using allele-specific PCR, 79 children with relapsing NS were genotyped for the IL12Bpro polymorphism, and genotype was correlated with clinical phenotype (presence/absence of steroid dependence).

**Results.** Children with the steroid-dependent course are at a significantly higher frequency homozygous for one IL12B allele compared to children without steroid dependence (46.7% and 17.6%, respectively). This genotype has previously been shown to be associated with impaired IL-12 secretion.

**Conclusion.** Polymorphisms in the IL12B promoter region associate with two different clinical courses of NS. The IL12Bpro polymorphism may therefore define molecular subgroups with different prognosis. Further studies are needed to evaluate the prognostic value.

**Keywords:** child; interleukin 12; nephrotic syndrome; Th1; Th2

Introduction

The pathogenesis of the idiopathic nephrotic syndrome (INS), especially of its most frequent form, steroid-sensitive nephrotic syndrome (SSNS), remains unclear. SSNS is clinically defined by proteinuria, hypoalbuminaemia, hyperlipidaemia and oedema; histopathological examination of kidney biopsies usually reveals minimal glomerular changes. Most patients with idiopathic nephrotic syndrome (INS) respond to steroid therapy and show a favourable outcome; however, patients who develop steroid dependence (i.e. relapses during or shortly after steroid treatment) often have a treatment refractory course, requiring intensified immunosuppression with cyclophosphamide and/or cyclosporine A [1,2].

A T-cell-dependent course is suggested by the detection of increased T helper 2 (Th2), T-cell-derived cytokines like interleukin 4 (IL-4) [3] and interleukin 13 (IL-13) [4] in acute relapse of INS. It is not yet clear whether these observations are indicative of a cause or a result of the disease.

Many authors have found an association of childhood INS with prototypical Th2-biased diseases (such as asthma and hay fever) or other evidence of a Th2 bias [3,5–7]. There has been a controversial discussion about the relevance of this phenomenon during the past decades that even involved therapeutic trials with desensitization. Serum IgE levels were measured in some of these studies but the results remained inconclusive [8,9].

Since interleukin 12 (IL-12) is an important inducer of interferon γ (IFN-γ) production and contributor to the Th1 phenotype, differences in the production of IL-12 might affect the clinical outcome of the disease. On the other hand, IL-12 has been implicated in the pathogenesis of INS since it is found elevated in serum of patients in relapse of SSNS and believed to upregulate the production of a vascular permeability factor [10].

IL-12 is produced by antigen-presenting cells, especially dendritic cells (DC), and regulates growth and development of both NK and T cells. It is the major inducer of IFN-γ
and therefore important in the generation of a Th1-biased immune response [11]. Recently, the association between a complex bi-allelic polymorphism [12,13] in the promoter region of the gene coding for the heavy chain of IL-12 (IL12Bpro) and the secretion of IL-12 has been described. DC from IL12Bpro allele 1 homozygous individuals produced significantly less IL-12 p70 in response to CD40 ligation as compared to DC from heterozygous or allele 2 homozygous individuals [14].

We hypothesized that a genetic deficiency in IL-12 production could result in a Th2-biased immune response and in turn predispose to specific clinical characteristics in SSNS. We therefore examined the frequency of the functional IL12B promoter polymorphism with regard to the clinical outcome and atopic status of children with SSNS.

Subjects and methods

Seventy-nine patients were identified who exhibited relapsing SSNS according to the criteria of the ‘International Study of kidney diseases in children’ and of the ‘Arbeitsgemeinschaft Pädiatrische Nephrologie’ [15,16]. Patients were subdivided according to their clinical course: steroid-dependent nephrotic syndrome (SD) was defined according to the APN with at least 2 relapses or on or within 14 days of discontinuation of alternate day prednisone (40 mg/m²/48 h) or non-steroid dependence (occasional, infrequent relapses or frequent relapses >4 relapses per year, or 2 in the first 6 months following presentation) but without SD.

Treatment of initial episode and relapses was according to the standard regimen [17]. At initial presentation prednisone is administered at a dose of 60 mg/m²/day for 6 weeks followed by 40 mg/m²/48 h for 6 weeks. Relapses are treated with prednisone 60 mg/m²/day until the urine is negative for protein for 3 days, followed by 40 mg/m²/48 h for 4 weeks.

DNA was extracted from EDTA blood (Qiagen blood extraction kit, Hilden, Germany). The detection of the IL12Bpro polymorphism has been described in detail previously [14]. The longer allele is referred to as IL12Bpro-1 and the shorter allele as IL12Bpro-2. The analysis in these subjects of polymorphisms in other cytokine genes (i.e. position 50 (Ile50/Val) and 551(Gln551/Arg) of the IL4 receptor and position 110 (Arg110/Gln) of IL-13 has been published previously [18].

Genotype frequencies of the polymorphisms were compared, and statistical analysis was performed with the SigmaStat software (Chicago, IL, USA). The study was approved by the local ethics committees. Controls were derived from normal blood donors and have been previously published [14].

Results

Seventy-nine children with relapsing SSNS (49 males and 30 females) were investigated. The mean age of the children was 10.7 ± 4.5 years. Thirty-four children were classified as non-SD and 45 as SD. All patients had received renal biopsies for histological diagnosis, showing minimal change nephrotic syndrome with no deposition of immunoglobulins and no signs of focal sclerosis.

The allele frequency in all patients of allele 1 was 0.54 and of allele 2 was 0.46 and not significantly different to previously published reports on the allele frequency [12–14]. Analysis of the clinically distinct subgroups showed that the allele frequency of IL12Bpro-1 was 0.61 in the SD patients and 0.45 in the group of non-SD patients, while IL12Bpro-2 frequency was 0.39 in SD patients and 0.55 in non-SD. Thus, the IL12Bpro-1.1 genotype was significantly more common in SD than in non-SD (Fisher’s exact test, P = 0.016 (two-tailed), Table 1). Thus, the hypothesis that IL12Bpro genotype is related to the clinical course of SSNS was confirmed.

Next we compared the IL-12 allele with the previously analysed data of the IL-4 receptor and IL-13 gene polymorphisms of 57 children. Interestingly, regardless of the clinical course, children with an IL12Bpro-1.1 genotype showed less Th2-biasing polymorphisms in both genes than those with an IL12Bpro-1.2 or IL12Bpro-2.2 genotype (Table 2).

Discussion

While mutations in genes coding for proteins involved in the glomerular filtration apparatus are increasingly being recognized as the cause for steroid-resistant nephrotic syndrome, the pathogenesis of steroid-sensitive nephrotic
syndromes is still unclear. An association with a Th2-biased immune response (defined as history of allergic disease or increase of Th2 cytokines and IgE) has been noted previously although analyses at the molecular level have been somewhat inconclusive. Since a Th2 immune response might be the default pathway in the absence of Th1-biasing cytokines, we decided to investigate the impact of a functional polymorphism in the IL12B gene on steroid-sensitive minimal change nephrotic syndrome. IL-12 is a major inducer of IFN-γ production and a major contributor to the Th1 phenotype; differences in the production of IL-12 might therefore be associated with either disease or clinical course of the disease.

The distribution of the genotypes in the total population of patients with SSNS was not significantly different to the normal population. This finding was not surprising since SSNS may be a molecularly heterogeneous disease with variable clinical course.

Upon analysis of the clinically distinct subgroups of steroid-dependent versus non-dependent disease profound differences in the distribution became apparent. IL12Bpro-1.1 genotype frequency of allele 1 (low IL-12 production) was higher in steroid-dependent disease, while the frequent relapsing disease course showed a normal distribution. This suggests a relevance of the increased IL-12 production capacity for either disposition or clinical course of steroid-dependent INS. Patients with steroid dependence are clinically well characterized and are often treatment refractory, while those with infrequent and even frequent relapses often carry a good prognosis [19]. They especially differ in their reaction to treatment with low-dose cytotoxic drugs, since patients with frequent relapses without steroid dependence can be treated successfully while those with SD appear to have less benefit from cytotoxic drugs [19].

Concordant to our findings, another group [5] showed that IFN-γ production was decreased in patients with SSNS compared to normal controls. Since IL-12 is the main inducer of IFN-γ, the determining factor for low IFN-γ production may be the genetic disposition for lower IL-12 production. Steroid-dependent INS may thus be viewed as a Th2 disease arising from a lack of Th1-biasing cytokines.

Many studies have focused on the relationship between INS and atopy in different populations [7,9,20]. In our previous study, we did not find an association between polymorphisms in the IL-4 receptor, IL-13 and IgE-receptor and the clinical course of disease, but we found a tendency towards increases in these polymorphisms in children with INS and atopy as defined by the skin prick test, RAST or IgE [18]. To test whether there might be a bias towards Th2-prone polymorphisms in INS, we compared the presence of the IL12B promoter polymorphism with the presence of previously published polymorphisms of the IL-4 receptor and of IL-13 and found a bias of the IL12Bpro-2.2 genotype (high IL-12, Th1) towards Th2-prone polymorphisms in the IL-4 receptor and IL-13 polymorphism, which means, as soon as the IL12Bpro-1.1 genotype is absent (the low IL-12 genotype), a Th2-prone polymorphism of the IL4R or of IL13 or both are present.

To further elucidate the pathogenesis of SSNS, additional studies are now required to compare the impact of the IL12Bpro polymorphism on IL-12 and IFN-γ secretion in relapse and remission of nephrotic syndrome.

In summary, these data suggest that childhood SSNS is a heterogenous disease but that the common denominator may be a Th2-skewed immune response caused either by a lack of Th1 or an increase of Th2-biasing cytokines. To our knowledge this is the first report to investigate simultaneously genetic parameters of both prototypical immune reactions.

Acknowledgement. The expert technical assistance of Ms Bettina Hill is gratefully acknowledged.

Conflict of interest statement. None declared.

References


Received for publication: 11.10.07
Accepted in revised form: 20.6.08