Psoriasis Is Not Associated with IL-12p70/IL-12p40 Production and *IL12B* Promoter Polymorphism

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Psoriasis is a type-1 T cell-mediated, chronic inflammatory disease. Since interleukin (IL)-12p70 promotes the development of type-1 T cells, we investigated whether psoriasis is associated with an increased production of this cytokine by blood cells. Results revealed that the production of IL-12p70 by cells of psoriasis patients stimulated by 1 and 10 ng per mL, but not 100 ng per mL of lipopolysaccharide (LPS) was higher ($p = 0.03$) than that by cells of healthy volunteers. The production of IL-12p40 by patients cells upon stimulation with 0.1 ng per mL LPS, but not higher concentrations, was higher ($p = 0.02$) than that by cells of healthy volunteers. No association between IL-12p70 production by blood cells and the severity of psoriasis was observed, nor was there a difference in the LPS-stimulated production of this cytokine between cells of the early and late onset type of patients. The frequencies of the various genotypes for the promoter region of the gene encoding IL-12p40 (*IL12B*) did not differ between psoriasis patients and controls. No association was observed between the various *IL12B* promoter genotypes and the LPS-stimulated production of IL-12p70 or IL-12p40 by blood cells. Together, psoriasis is not associated with a promoter polymorphism in the *IL12B* gene nor with the production of IL-12p70 by LPS-stimulated blood cells.

Key words: blood cells/IL12B promoter polymorphism/interleukin-12/psoriasis
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Psoriasis vulgaris is a type-1 T cell-mediated chronic skin disease characterized by epidermal hyperplasia and infiltration of inflammatory cells into the affected skin (Uyemura *et al*, 1993; Nestle *et al*, 1994; Schlaak *et al*, 1994; Nickoloff, 1999). Based on the age at onset of the disease, two types of psoriasis can be discriminated, the early onset (age at onset <40 y) affecting about 75% of the psoriasis patients and the late onset (age at onset ≥ 40 y) occurring in approximately 25% of the cases (Stuart *et al*, 2002). It has been reported that type-1/pro-inflammatory cytokines are predominantly expressed in affected skin lesions (Bos and De Rie, 1999) and circulation (Austin *et al*, 1999). IL-12p70, an important pro-inflammatory cytokine mainly produced by antigen presenting cells, is believed to play a pivotal role in inducing type-1 cytokine profiles. This cytokine is a heterodimer consisting of the IL-12p35 subunit, which is constitutively expressed and the IL-12p40 subunit, whose expression is inducible. The two genes for these subunits, *IL12A* encoding IL12-p35 located on 3p12–q13.2 and *IL12B* encoding IL-12p40 located on 5q31—33, are unrelated (Warrington and Bengtsson, 1994; Trinchieri, 2003).

Polymorphisms in cytokine or cytokine receptor genes are associated with susceptibility to auto-immune, inflammatory, and infectious diseases (Hall *et al*, 2000). Recently, Morahan *et al* (2002) found an association between IL-12p70 production, *IL12B* mRNA expression and a promoter polymorphism in the *IL12B* gene, and the severity of asthma. In earlier studies, polymorphisms in IL-10, tumor necrosis factor (TNF)-α, and IL-1β were found to be associated with psoriasis (Asadullah *et al*, 2001; Reich *et al*, 2002). Tsunemi *et al* (2002) found an association between a single-nucleotide polymorphism (SNP) in the 3′-untranslated region (3′-UTR) of the *IL12B* and the susceptibility to psoriasis vulgaris. As psoriasis is known to be a type-1 cytokine-mediated disease and the promoter polymorphism described by Morahan *et al* (2002) was functionally linked to IL-12p70 production, the aim of this study was to determine whether psoriasis is associated with an increased production of IL-12p70/p40 by lipopolysaccharide (LPS)-stimulated blood cells and whether psoriasis is associated with a polymorphism in the *IL12B* promoter region.

Abbreviations: IL, interleukin; LPS, lipopolysaccharide

Results

As psoriasis is believed to be a type-1 mediated disease, we first analyzed whether production of IL-12p70 and IL-12p40 differed between psoriasis patients and healthy controls. For this purpose, we determined the production of IL-12p70 and IL-12p40 by whole blood cells in response to increasing doses of LPS. The results revealed that doses of 1 and 10 ng per mL, but not 100 ng per mL, of LPS resulted in a significantly higher ($p = 0.03$) production of IL-12p70 (Fig 1*A*) by blood cells of psoriasis patients, i.e., 9 ± 3 and 17 ± 4 pg per mL for 1 and 10 ng per mL LPS, respectively,

as compared to blood cells of healthy volunteers (1 ± 1 and 4 ± 2 pg per mL, respectively). The production of IL-12p40 by blood cells in response to 0.1 ng per mL LPS, but not higher doses of LPS, was significantly higher ($p = 0.02$) in psoriasis patients (1526 ± 451 pg per mL) than in healthy volunteers (422 ± 166 pg per mL).

In addition, the LPS-induced production of IL-12p70 and IL-12p40 by blood cells did not differ among the two onset types of psoriasis. Furthermore, no association between the severity of psoriasis, as assessed by the PASI-score, and the LPS-stimulated IL-12p70/p40 production by blood cells was found.

Stimulated by the report of an association between a promoter polymorphism in the *IL12B* gene and severity of asthma (Morahan *et al*, 2002), we investigated whether this promoter polymorphism was associated with the severity of psoriasis. The results revealed no differences in the distribution of the various genotypes of the *IL12B* promoter for psoriasis patients (Table I) when compared to the distribution of the various *IL12B* genotypes in the control group (Table I) and the earlier reported distributions (Morahan *et al*, 2002). Moreover, no association between this promoter polymorphism and the severity of psoriasis was observed. In addition, no differences in the frequencies of the *IL12B* promoter genotypes in the two types of psoriasis were observed.

Next, we collectively analyzed the production of IL-12p70 and IL-12p40 of the psoriasis patients and healthy volunteers to investigate whether these values were associated with the various *IL12B* genotypes. In agreement with the above data, no relation between the production of IL-12p70 (Fig 2*A*) and IL-12p40 (Fig 2*B*) and the genotype for the *IL12B* promoter was observed.

Discussion

The first conclusion to be drawn from the present results is that the LPS-stimulated IL-12p70 and IL-12p40 production by blood cells is not associated with psoriasis. In agreement, a previously published report showed that the serum levels of IL-12p70 in psoriasis patients and healthy volunteers do not differ (Economidou *et al*, 1999). The above conclusion is based on the following findings. First, blood cells of psoriasis patients did not produce more IL-12p70 or IL-12p40 upon stimulation with 100 ng per mL (but not lower) doses of LPS than cells of healthy volunteers did. Second, no association was observed between the severity of disease and the LPS-stimulated IL-12p70 or IL-12p40 production by blood cells. Furthermore, we could not find a difference between the two types of onset of psoriasis with respect to the LPS-stimulated production of IL-12p70 and IL-12p40 by blood cells. But it should be realized that blood cells of psoriasis patients did produce more IL-12p70/IL-12p40 than blood cells of healthy volunteers upon stimulation with suboptimal doses of LPS. These data may indicate that patients cells are "primed to a type-1 immune response".

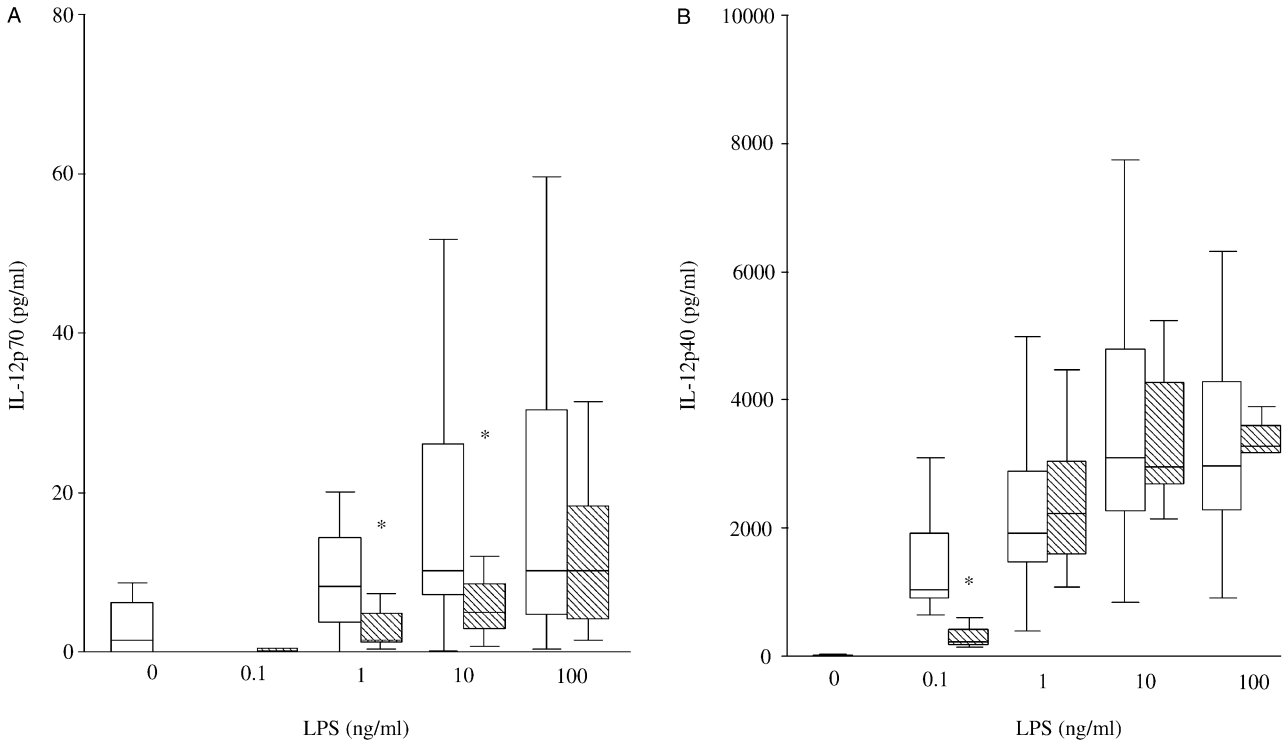

Figure 1
Interleukin (IL)-12p70 (*A*) and IL-12p40 (*B*) production by lipopolysaccharide (LPS)-stimulated blood cells of psoriasis patients and healthy volunteers. In short, blood was diluted four times with medium and then LPS (range 0.1–100 ng per mL) was added. After 24 h incubation at 37°C, the cells were centrifuged and the supernatants were analyzed for the presence of IL-12p70 (*A*) and IL-12p40 (*B*) by ELISA's. Results are from 22 psoriasis patients (open boxplots), either receiving no therapy or topical therapy, and from 11 healthy volunteers (hatched boxplots). The top, bottom, and middle lines correspond to the 75th percentile, 25th percentile, and median, respectively. Bars extend to the top 90th percentile and to the 10th percentile of each group. *Indicates values significantly different ($p < 0.05$) from cells of healthy volunteers.

Table I. Genotype frequencies of the interleukin *(IL)12B* promoter in psoriasis patients and controls

IL12B genotypes	Psoriasis patients			Controls
	Early onset (n = 27)	Late onset (n = 10)	Total (n = 37)	(n = 131)
1.1	5 (0.2)[a]	3 (0.3)	8 (0.2)	37 (0.3)
1.2	15 (0.5)	6 (0.6)	21 (0.6)	66 (0.5)
2.2	7 (0.3)	1 (0.1)	8 (0.2)	28 (0.2)

[a]The numbers in parentheses indicate the ratio between number of patients with a certain genotype and the total number of patients in a group. Similarly, ratios were calculated for the controls.

The second conclusion pertains to the frequencies of the various genotypes of the *IL12B* promoter in psoriasis patients. Our results revealed that the frequencies of the various *IL12B* promoter genotypes in psoriasis patients were not different from that reported for healthy volunteers (Morahan *et al*, 2002). In addition, the frequencies of the various *IL12B* promoter genotypes did not differ among the two types of onset of psoriasis. This is in contrast to other cytokines, such as IL-1β, TNF-α and IL-10, in which particular genotypes are associated with the two types of onset of psoriasis (Asadullah *et al*, 2001; Reich *et al*, 2002). No association between the *IL12B* promoter genotypes and the severity of psoriasis was found. It should be noted that Tsunemi *et al* (2002) reported an association between an SNP within *IL12B* 3′-UTR and susceptibility to psoriasis. That 3′-UTR was not functionally linked to production of IL-12p70 or IL-12p40 whereas the *IL12B* promoter polymorphism (Morahan *et al*, 2002) was. We were, however, not able to confirm such an association between the production of IL-12p70 or IL-12p40 by LPS-stimulated blood cells and the genotype for the *IL12B* promoter in psoriasis patients or healthy volunteers. This might be due to differences in the genetic background between our subjects and those described in the paper by Morahan *et al* (2002).

Finally, it should be realized that this study was performed with blood cells, whereas the local production of IL-12p70 by antigen presenting cells in the psoriatic skin may be enhanced as compared to non-psoriatic, inflamed skin (Cheng *et al*, 2001). Nevertheless, our results indicate that IL-12 production may not be the key factor involved in the pathogenesis of psoriasis patients. Therefore, research should be focussed on the roles of T cells and their cytokines in the pathogenesis of psoriasis; however, the observation that suboptimal doses of LPS induced a higher production of IL-12p70 and IL-12p40 by circulating cells of psoriasis patients as compared to those of healthy volunteers supports the hypothesis that psoriasis is a type-1 cytokine-mediated immune disease.

Materials and Methods

Subjects This study was approved by the medical ethical committee of the Leyenburg hospital (MEC number: 95–719, The Hague, The Netherlands) and after obtaining written informed consent, conducted in 38 psoriasis patients (18 men and 20 women). Seventeen patients received no anti-psoriasis therapy at

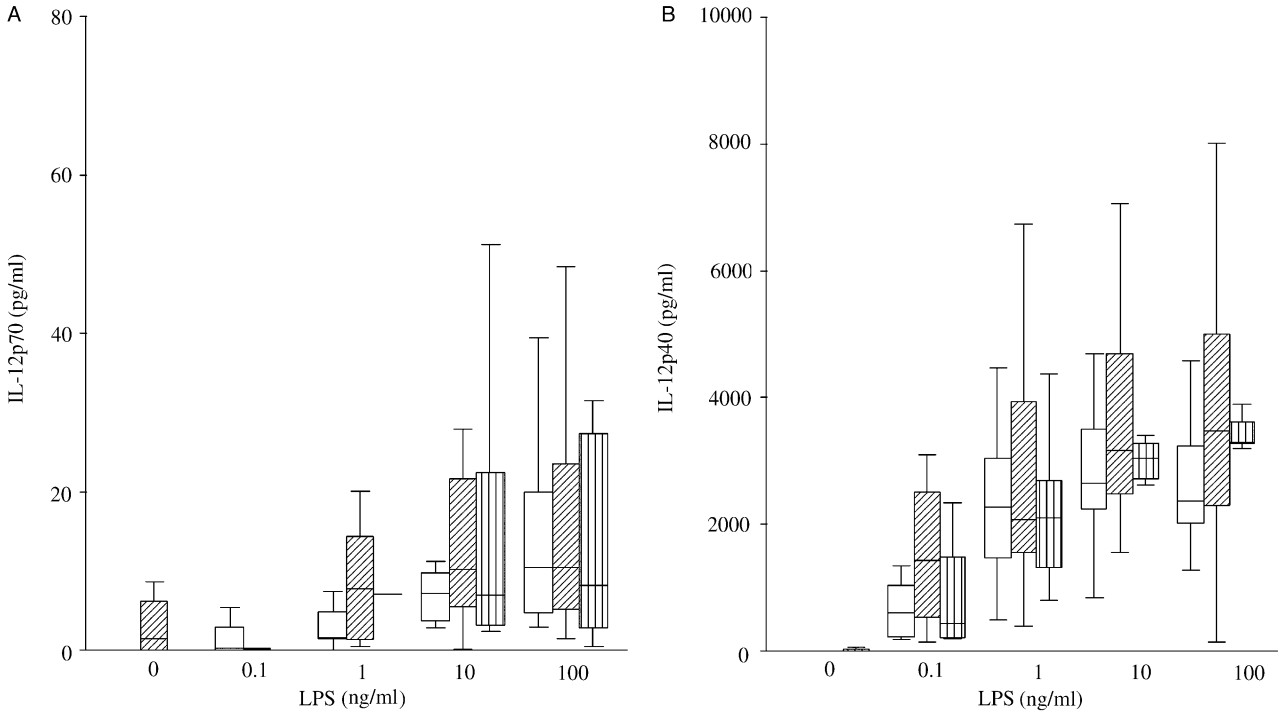

Figure 2
Interleukin (IL)-12p70 (*A*) and IL-12p40 (*B*) production by lipopolysaccharide (LPS)-stimulated blood cells of patients and healthy volunteers with the different IL12B promoter genotypes. In short, blood was diluted four times with medium and then LPS (range 0.1–100 ng per mL) was added. After 24 h incubation at 37°C, the cells were centrifuged and the supernatants were analyzed for the presence of IL-12p70 (*A*) and IL-12p40 (*B*) by ELISA's. Results are from eight individuals with the 1.1 genotype (open boxplots), 15 individuals with the 1.2 genotype (hatched boxplots) and eight individuals with the 2.2 genotype (vertically hatched boxplots) for the IL12B promoter gene.

the moment of inclusion in the study, seven were treated topically and 14 were treated systemically. Ten of the 38 patients (26%) (three men and seven women) developed psoriasis at late age and 28 (74%) (15 men and 13 women) developed this disease at an early stage of life. The mean ages at onset for the two types of psoriasis patients in this study were 49 ± 3 and 16 ± 3 y, respectively. The mean psoriasis area and severity index (PASI)-scores were almost identical. They were 11 ± 2 and 10 ± 1 for the early and late onset of psoriasis, respectively. As controls, 44 healthy volunteers were included in this study and for the *IL12B* gene frequency studies the DNA of 131 patients undergoing cardiac surgery (Bouter *et al*, 2002) were analyzed. For this study, approval of the medical ethical committee of the Leiden University Medical Center (MEC-number:P168/96, Leiden, The Netherlands) as well as written informed consent by the controls was obtained.

Blood was drawn into endotoxin-free tubes (Chromogenix Endotube, Mölndal, Sweden) for assessment of IL-12p70 and IL-12p40 production by circulating leukocytes and the remaining blood was used for extraction of DNA and subsequent determination of the *IL12B* promoter genotype.

Whole-blood stimulation and ELISA A whole-blood stimulation was performed to determine the cytokine production by blood cells. Briefly, blood was diluted four times in RPMI-1640 (Gibco BRL, Paisley, Scotland, UK) and then incubated for 24 h at 37°C with medium only or medium containing LPS of *Escherichia coli* O111:B4 (0.1, 1, 10, or 100 ng per mL; Difco Laboratories, Detroit, Michigan). Subsequently, the supernatants were collected and stored at −70°C until further analysis.

The amounts of IL-12p70 and IL-12p40 were determined in these supernatants using commercial ELISAs (IL-12p70; Elipair, Diaclone Research, Besançon, France and IL-12p40; Biosource Europe SA, Fleurus, Belgium) following manufacturer's instructions. Each sample was assayed in duplicate. The detection limits were 6 and 31 pg per mL, respectively.

DNA extraction and *IL12B* promoter polymorphism To assess the genotype for the *IL12B* promoter, the following protocol derived from that described by Morahan *et al* (2002) was followed. In short, DNA was isolated from blood after lysis of erythrocytes using the Wizard® Genomic DNA Purification kit (Promega, Madison, Wisconsin) according to manufacturer's instructions. We amplified DNA samples with two primers located in the *IL12B* promoter region. The sequences of the primers (Isogen Bioscience, Maarssen, The Netherlands) are: IL12B-1188 forward 5′-TTTGGAGGAAAAGTGGAAGA-3′; IL12B-1188 reverse 5′-AA-CATTCCATACATCCTGGC-3′.

PCR reactions were performed in a final reaction volume of 50 μL PCR buffer (containing 2.5 mM $MgCl_2$) using 100 ng of DNA, 10 pmol of each primer, 0.5 mM dNTPs and 0.8 U Amplitaq DNA polymerase (Perkin–Elmer, Branchburg, New Jersey). The PCR reaction consisted of one denaturation step of 3 min at 95°C, and subsequently the PCR was done for 30 cycles: 20 s at 95°C followed by 20 s at 55°C and 30 s at 72°C with a final 2 min extension at 72°C. PCR products were detected after separation on a 2% agarose gel (Promega).

To type/analyze the polymorphism consisting of a 4 base-pair insert, we used a restriction enzyme which cuts the allele that contains the insert. For this purpose, 8 μL of the PCR product were incubated for 16 h at 37°C with 0.3 U *Bpl* I (MBI Fermentas, St. Leon-Rot, Germany) in the presence of 2.5 mM S-adenosylmethionine in Y+/Tango™ buffer (MBI Fermentas). The product was subsequently run on a 10% polyacrylamide gel to visualize the two alleles. The insert allele was designated "allele 1" and the shorter allele was termed" "allele 2"; the various genotypes are referred to as 1.1, 1.2, and 2.2.

Statistical analysis Statistical analyses were performed using SPSS for Windows version 11.0. A univariate analysis was used to determine whether variations in circulating levels of IL-12p70 and IL-12p40 could be attributed to being a patient or control and to having an early or late onset type of psoriasis. Comparison of the distribution of the genotypes for the *IL12B* promoter were performed using the χ^2-square test. In addition, we also analyzed whether the production of IL-12p70 and IL-12p40 were related to the genotype of the *IL12B* promoter using a univariate analysis. Results are means ± SEM.

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