Individual susceptibility to chronic irritant contact dermatitis

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Polymorphisms in the interleukin-1 gene influence the stratum corneum interleukin-1α concentration in uninvolved skin of patients with chronic irritant contact dermatitis

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Abstract

Background  Interleukin (IL)-1α and its receptor antagonist IL-1RA, play a role in skin inflammation. Several polymorphisms in the IL1 gene cluster, coding for IL-1α, IL-1RA and IL-1β, influence their protein expression. Within this cluster, strong linkage disequilibrium has been shown.

Objectives  We studied the association between the polymorphisms IL1A -889 (C→T) and IL1B -31 (T→C) and the concentration of IL-1α and IL-1RA in the stratum corneum (SC).

Methods  In 124 patients with chronic irritant contact dermatitis, we genotyped the IL1A -889 and IL1B -31 polymorphisms and determined the amount of IL-1α and IL-1RA on tape strips obtained from uninvolved skin of the volar forearm.

Results  The SC IL-1α concentration was 23% and 47% lower in subjects with IL1A -889 C/T genotype and T/T genotype, respectively, compared with wildtype genotype. In subjects with IL1B -31 C/C genotype, the IL-1α concentration was 51% lower compared with C/T and T/T genotype. The ratio IL-1RA/IL-1α increased twofold in IL1A -889 C/T genotype and threefold in T/T genotype compared with wildtype.

Conclusions  We showed a clear effect of IL1 genotype on protein expression in the SC. This altered expression may be responsible for the interindividual differences in the inflammatory response of the skin.
**Introduction**

Chronic irritant contact dermatitis (CICD) is a frequently occurring occupational disease, mainly caused by repetitive contact of the skin to irritants and most often located on the hands and forearms. The eczematous reaction is characterized by dryness, redness, scaling and hyperkeratosis.\(^1\) The mechanism of the development of CICD and the predisposing factors for this skin disease are not well understood. Cytokines and chemokines play an important role in skin inflammation by orchestrating cutaneous inflammatory and repair processes.\(^2\) Upon an external insult, keratinocytes produce a wide variety of inflammatory mediators, e.g. GM-CSF, IFN-\(\gamma\), IL-1\(\alpha\), IL-1\(\beta\), IL-6, IL-8 and TNF-\(\alpha\), resulting in a pro-inflammatory cascade reaction.\(^2,3\) To counteract this inflammatory response, also anti-inflammatory cytokines are produced, e.g. IL-1RA and IL-10.\(^2,4\) The balance between pro- and anti-inflammatory cytokines is important for the extent and duration of the individual inflammatory response.\(^2\)

In genes encoding cytokines, several polymorphisms were found, which influence the levels of these proteins.\(^5\) These genetic variants might play a role in individual susceptibility to skin inflammation. Polymorphic loci in cytokine genes might be associated with altered levels of cytokines by directly affecting gene transcription. Gene polymorphisms can also be non-functional but can still be associated with altered protein expression due to linkage disequilibrium (LD) with a functional polymorphism in the same or even adjacent genes.\(^6,7\)

In the literature, the *IL1A* -889T allele has been associated with several inflammatory diseases including juvenile rheumatoid arthritis, periodontitis, and Alzheimer’s disease.\(^8\) Other studies have shown no associations\(^8,9\) or reported a protective effect of the *IL1A* -889T allele on for example systemic lupus erythematosus\(^10\) and systemic sclerosis.\(^11\) The *IL1A* -889 polymorphism is located in the promoter region, which may alter transcriptional activity of the *IL1A* gene.\(^12\) Data on the *in vivo* influence of the *IL1A* -889T allele on protein expression is still limited. In patients with severe periodontal disease, the *IL1A* -889T allele was associated with increased IL-1\(\alpha\) protein levels in gingival crevicular fluid.\(^13\)

To find out whether this *IL1A* -889 single nucleotide polymorphism (SNP) is associated with altered cutaneous IL-1\(\alpha\) protein expression, we studied the influence of this SNP on IL-1\(\alpha\) concentration in the stratum corneum (SC). IL-1\(\alpha\) is constitutively produced by keratinocytes and the SC has been shown to act as a major reservoir of active IL-1\(\alpha\).\(^14\) Genetic studies showed LD between several polymorphic loci within the *IL1* gene cluster, which contains the genes *IL1A*, *IL1B*, and *IL1RN*.\(^15,16\) As the *IL1B* -31 polymorphism is in LD with other poly-
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morphisms within the *IL1A* gene,\textsuperscript{16} also a possible influence of this SNP on the SC IL-1α concentration was studied. Besides the cytokine IL-1α, also IL-1RA is abundantly present in the SC.\textsuperscript{17,18} The *IL1RN* gene, coding for IL-1RA, is also located within the *IL1* gene cluster and therefore we studied whether the *IL1A* -889 and *IL1B* -31 SNPs were associated with an altered SC IL-1RA concentration. IL-1RA exerts an anti-inflammatory function by blocking IL-1 activity by competitive binding to the IL-1 receptors without triggering a signal cascade.\textsuperscript{19} Therefore, we also studied the balance between the cytokine IL-1α and its antagonist IL-1RA.

In this study, we obtained SC tape strips from uninvolved skin of the volar forearm of 124 patients with CICD and determined the amount of IL-1α, IL-1RA and soluble protein on the tapes. The *IL1A* -889 and *IL1B* -31 genotypes were determined using DNA isolated from buccal swab samples obtained from each subject.

**Methods**

**Subjects**

SC tape strips were collected from 124 CICD patients, registered at the Department of Dermatology, University of Osnabruceck in the months November until May. The patients had CICD of the hands during ≥ 3 months at examination or had had medically verified CICD of the hands during at least 1 period of ≥ 3 months (CICD was mostly occupational). For each patient, a dermatologist completed a check list on diagnosis, course of the disease, treatment, patch test results, and occurrence of atopic diseases in the past and present. The patients were classified using the following three diagnoses regarding the hands (i) CICD without atopy, i.e. erythema, scaling and fissures, located at the back of the hands or the interdigital web spaces, strictly exposure-dependent course and no signs of atopy, (ii) CICD with atopic skin disposition, i.e. clinical features as (i) but additionally major atopic signs (flexural eczema) or combinations of so called ‘minor criteria’ of skin atopy,\textsuperscript{20-22} and (iii) irritant-induced atopic eczema, i.e. predominantly palmar itch, vesicles, erythema, oozing, symmetrically present, protracted course after exposure discontinuation.\textsuperscript{23,24} Patients with other chronic inflammatory diseases (i.e. rheumatoid arthritis, Crohn’s disease, systemic lupus erythematosus, and psoriasis) were excluded. All included patients were Caucasian and ≥ 18 years. Written informed
consent was obtained from each subject before entering the study. The study was approved by the Ethical Committee of the University of Osnabrueck.

Tape stripping

Tape strips were taken from the volar forearm on skin free from visible eczematous lesions. Templates of Scanpor® tape (Norgeplaster, Vennesla, Norway) were fixed to the skin around each site to limit the tape stripping area to a circular area of 18-mm diameter. Four strips were taken using Diamond® adhesive tape (Diamond Ultra Clear Tape, 19 mm x 25 mm; The Sellotape Company, Eindhoven, the Netherlands). The four pieces of tape were successively applied to the sites and homogeneously pressed on to the skin by moving a 1.0-kg stainless steel roller to and fro ten times. The tapes were then quickly removed at an angle of 170° with the skin. During the tape stripping procedure, the examiner wore vinyl-gloves and used forceps. The first tape was discarded and the following three tapes were pooled into a 20 ml glass vial. The vials were immediately stored at -18 °C until extraction.

The amount of soluble protein and cytokines was determined based on a generally used approach as described previously. In short, two ml phosphate-buffered saline (Merck, Darmstadt, Germany) with 0.005% Tween-20 (Sigma-Aldrich, Zwijndrecht, the Netherlands) was added to each vial, and the vials were left on ice for 30 min. Extraction was performed using an ultrasound sonifier equipped with a probe (Salm & Kipp, Breukelen, the Netherlands) for 15 min in ice-water. The extract was centrifuged (1 min, 15000 g) and supernatant aliquots were re-frozen at -80 °C until required for further analysis. The extracts were analysed for the cytokines IL-1α and IL-1RA using specific enzyme-linked immunosorbent assay (ELISA) kits (Human CytoSets, Biosource International, Camarillo, CA, USA). The amount of soluble protein in each extract was determined with the Micro BCA protein assay kit (Pierce, Rockford, IL, USA) using the supplied bovine serum albumin as standard. No IL-1β can be measured using the non-invasive tape stripping approach.

DNA sampling and isolation

For collection of DNA material, buccal mucosa cells were used obtained by rubbing the inside of the cheeks with a cotton swab on a plastic stick (Medispo, Oud Beijerland, the Netherlands). After the mouth was rinsed with water, two swabs were obtained. After sampling, each swab was placed into a 15 ml tube (Greiner Bio-one, Alphen a/d Rijn, the Netherlands) with 2 ml of lysis buffer (Puregene® Cell Lysis Solution, Gentra Systems, Minneapolis, MN, USA)
to disrupt the cells and stabilize the DNA. Genomic DNA was extracted using a commercial DNA isolation kit (Puregene®, Gentra Systems, Minneapolis, MN, USA) based on a standard proteinase K digestion method according to the manufacturer’s protocol. The tubes were stored at 4 °C up to 3 months before DNA isolation. The amount of DNA was quantified by absorbance at 260 nm, and an aliquot was diluted to a working concentration of 2-10 ng/μl.

Genotyping
Genotyping for IL1A -889 (C→T) (rs1800587) and IL1B -31 (T→C) (rs1143627) polymorphisms was performed by means of a fluorogenic 5’ nuclease PCR TaqMan® assay (Applied Biosystems, Foster City, CA, USA) using reagents obtained from Applied Biosystems (Primer/Probe Mix and TaqMan® Universal PCR Master Mix without AmpErase® UNG) and following the manufacturer’s protocol. Analyses were made on an ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA). Each plate contained two non-template controls and five allelic controls. Random replicate samples were determined (10% of all samples); intrasubject concordance rates of this method was shown to be >99% in a larger study.28

Statistics
The observed genotype frequencies were compared with the expected Hardy-Weinberg distribution by χ² test. LD was examined using Haploview software (version 3.32, Cambridge, MA, USA). Statistical analyses were performed using Pearson’s correlation coefficient, Student’s t-test and one-way analysis of variance (ANOVA) with LSD (pairwise comparison) post hoc test. Data on cytokine concentrations were log-transformed to obtain normal distributions. Accordingly, geometric means (meanG) and geometric standard deviations [SDG] were used. The statistical analysis was performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).
**Results**

Tape strips were sampled from 53 males (aged 42 ± 10 years; mean ± SD) and 71 females (aged 38 ± 13 years). 35% of the patients were diagnosed as CICD without atopy, 18% as CICD with atopic skin disposition and 47% as irritant-induced atopic hand eczema. Of all patients, 55% had one or more type IV sensitizations. The genotype distribution for **IL1A -889** was: C/C 48%, C/T 40% and T/T 12% and for **IL1B -31**: T/T 48%, T/C 39% and C/C 13%. Genotype distributions did not deviate from Hardy-Weinberg equilibrium for both polymorphisms and there was no LD between these two SNPs (D’ = 0.352; LOD = 1.05). Genotype distributions were closely similar from the point of view of sex, age, sub-diagnosis and type IV sensitizations.

The concentration of IL-1α and soluble proteins were analysed in the SC tape strips of all 124 subjects, and the concentration of IL-1RA was analysed in the tapes of 74 subjects. On average, tape strips contained 8.3 [2.6] pg IL-1α/μg protein (mean [SD]) and 6.7 [4.2] pg IL-1RA/μg protein, the ratio between IL-1RA and IL-1α was 0.7 [4.1]. The correlation between IL-1α and IL-1RA was 0.39 (P < 0.001). No differences were found in the IL-1α concentration between males (8.7 [2.5]) and females (8.1 [2.7]; P > 0.2), but the IL-1RA concentration was lower in males (4.8 [4.0]) compared with females (8.8 [4.2]; P = 0.049), as well as the ratio between IL-1RA and IL-1α (0.5 [4.1] vs. 0.9 [3.9]; P = 0.042). Given the equal distribution of genotypes regarding sex, sex is not a confounder of the relation between **IL1** genotypes and the cytokines IL-1α and IL-1RA. No differences in cytokine concentrations were found between the three sub-diagnosis groups or between patients with and without type IV sensitizations (data not shown).

The SC IL-1α concentration in subjects with **IL1A -889** C/C genotype was 10.0 [2.5] pg/μg protein and showed a genotype dependent decrease to 7.7 [2.8] pg/μg protein in subjects with C/T genotype (Figure 1A) and to 5.3 [2.3] pg/μg protein in subjects with T/T genotype (P = 0.023). The SC IL-1α concentration was also influenced by **IL1B -31** genotype (Figure 1B). The IL-1α concentration was 9.2 [2.5] and 9.1 [2.7] pg/μg protein in subjects with **IL1B -31** T/T and T/C genotype, respectively and decreased to 4.5 [2.2] in subjects with **IL1B -31** C/C genotype (P = 0.006 and P = 0.008, respectively).
Figure 1. The IL-1α concentration normalized by soluble protein obtained by stratum corneum tape strips for *IL1A*-889 genotype (A) and *IL1B*-31 genotype (B). One-way analysis of variance (ANOVA) with LSD (pairwise comparisons) *post hoc* test.

Whereas the SC IL-1α concentration showed an *IL1A*-889 genotype dependent decrease, the IL-1RA concentration showed an opposite, however not significant, trend. The SC IL-1RA concentration increased from 5.3 [3.8] pg/μg protein for C/C genotype to 7.6 [4.7] pg/μg protein for C/T genotype and to 12.3 [3.9] pg/μg protein in subjects with *IL1A*-889 T/T genotype. When examining the balance between IL-1RA and IL-1α, the IL-1RA/IL-1α ratio was 0.5 [2.6] in subjects with C/C genotype and showed a genotype dependent increase to 1.0 [4.5] in subjects with C/T genotype (*P* = 0.015) and to 1.6 [2.1] in subjects with T/T genotype (*P* = 0.021). No associations were found between *IL1B*-31 genotype and SC IL-1RA concentration or IL-1RA/IL-1α ratio.
Discussion

Using a SC tape stripping technique in CICD patients, we have shown that subjects with the variant *IL1A* -889T allele have a lower concentration of the pro-inflammatory cytokine IL-1α in the outermost layers of the skin as well as a higher IL-1RA/IL-1α ratio. The IL-1RA/IL-1α ratio, which partly determines the inflammatory response, was caused by a higher concentration of IL-1α but also by a lower IL-1RA concentration. The observed influence of *IL1A* genotype on SC IL-1α concentrations might not only be caused directly by a functional *IL1A*-889 polymorphism but also by other polymorphic loci within the *IL1A* gene considering the high degree of LD within this gene.15,16

For the *IL1B* -31 polymorphism, which is also located within the *IL1* gene cluster, we showed that the variant C/C genotype was associated with a lower SC IL-1α concentration. We found no clear LD between the two polymorphic loci *IL1A* -889 and *IL1B* -31, as also reported by others.16 However, also for the IL1B -31 SNP, it is imaginable that the association with IL-1α is caused through LD with another polymorphism in the *IL1A* gene.16 One may also speculate that the effect of *IL1B* -31 on IL-1α is mediated by the local concentration of IL-1β.

In a genetic association study, we observed that apprentices in vocational training for high-risk occupations for CICD with the variant *IL1A* -889T allele had a lower risk of symptoms of dermatitis at the hands.28 Regarding a lower concentration of the pro-inflammatory cytokine IL-1α and a favourable balance between IL-1RA and IL-1α in the SC, probably this *IL1A* -889T allele might be considered as a protective genotype against skin irritation.

Our study was the first to investigate the relation between genotype and cytokine expression in the skin. Our results with respect to IL-1α are opposite to what other investigators reported in other tissues. An *in vivo* study showed that the *IL1A* -889T allele was associated with increased IL-1α protein levels in gingival crevicular fluid of patients with severe periodontal disease.13 In an *ex vivo* experiment, an increased production of IL-1α was shown in LPS-stimulated peripheral blood mononuclear cells from subjects with an *IL1A* -889T allele.12 Further studies are needed to investigate the mechanism by which this polymorphism exerts different effects on IL-1α expression in different cell types and tissues.

We studied the relation between genotype and SC cytokine expression in patients with CICD. As the tape strips were obtained from non-involved skin of the volar forearm, we assumed that their state of disease does not affect the expression of IL-1α and IL-1RA. Indeed,
we found no relation between the Osnabrueck Hand eczema Severity Index (OHSI)\textsuperscript{29} as determined in the patients at the time of the investigation and the concentrations of IL-1\(\alpha\) and IL-1RA (data not shown). We suppose that the polymorphisms studied here in patients have a similar influence in healthy subjects. In our study, the concentrations of IL-1\(\alpha\) and IL-1RA in the upper layers of the SC were within the same range as we found previously.\textsuperscript{26} Sex-related differences in SC IL-1RA concentrations and in the ratio between IL-1RA and IL-1\(\alpha\) were not previously reported. Possibly, higher sun exposure in females may explain these differences, as UV-B light is known to increase IL-1RA and the ratio IL-1RA/IL-1\(\alpha\).\textsuperscript{27}

SC tape stripping is a relatively fast and simple technique. However, a limitation is that in normal healthy SC, only IL-1\(\alpha\) and IL-1RA are present in sufficient amounts for routine cytokine determination.\textsuperscript{17} To study relations between polymorphisms in genes encoding other cytokines and their level of expression in the skin, a different sampling technique is needed. Recently, a new technique for cytokine sampling has been introduced, which includes obtaining epidermis-derived fluid by a suction procedure after microporation of the SC by a laser device. By using this technique, we were able to determine more than 13 cytokines in epidermis-derived fluid obtained from normal, healthy skin.\textsuperscript{30} This device might be a good tool to study the effects of cytokine gene polymorphisms on the cytokine concentration in the viable epidermis.

This study is the first to show altered cutaneous protein expression among different cytokine SNP genotypes. We expect that further studies of such associations will lead to more insight in the mechanism by which genetic differences influence the susceptibility to skin inflammation and will open up perspectives for predictive testing in occupational dermatology.
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