Impact of tumour necrosis factor-α polymorphisms on irritant contact dermatitis

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Summary

Background. Genetic variations in genes coding for cytokines involved in skin inflammation may alter their expression, thus changing the susceptibility to irritant contact dermatitis (ICD).

Objectives. To determine the prevalence of polymorphisms in the cytokine genes TNFA-238 and TNFA-308 in patients with occupational ICD, and to compare it with that in controls.

Methods. In a case–control study, 478 patients with occupational ICD of the hands were genotyped for TNFA-238 and TNFA-308 polymorphisms. The results were compared with those of 393 apprentices from the same high-risk occupations (controls).

Results. For a carrier of a variant TNFA-238A allele, the odds ratio (OR) of acquiring ICD was 0.57 [95% confidence interval (CI) 0.34–0.97], suggesting a protective effect of the A allele. The genotype distributions were 94.4% wild type (G/G), 5.6% heterozygous (G/A) and 0% homozygous for variant allele (A/A) in patients, and 90.9%, 8.5%, and 0.6%, respectively in controls. In contrast, carriers of the variant TNFA-308A allele had an increased risk of ICD [OR 1.33; 95% CI 1.05–1.74; G/G 66.4%, G/A 31.2%, and A/A 2.4% (patients) versus 73.5%, 24.6%, 1.9% in controls].

Conclusions. Individuals with a TNFA-238 polymorphism are less prone and those with a TNFA-308 polymorphism are more prone to develop ICD of the hands, suggesting a protective versus a detrimental effect of the A allele respectively.

Key words: contact dermatitis; cytokines; tumour necrosis factor.

Irritant contact dermatitis (ICD) is a common inflammatory skin disorder and the leading occupational skin disease (1), affecting up to 10% of individuals in the general population in Europe (1-year prevalence) and 20% in ‘risk professions’ (2). The hands are the most commonly involved body site. As ICD is a preventable disease when eliciting irritants are avoided and appropriate protective measures are taken, early-stage identification is important.

The pathogenesis of ICD is multifactorial; both genetic and exogenous factors contribute to its development. Although skin exposure to irritants is a prerequisite for its development, there is substantial evidence that, with similar exposure, some individuals are more prone or less prone to acquire ICD than others. The most studied endogenous risk factor is atopy (3, 4), and even though it has also been proposed to aid in the identification of individuals at risk, its predictive value is controversial.

The question arises of further causes that may influence the development of ICD. As cytokines mediate and
regulate immunity and inflammation in the skin, genetic variations in genes coding for these cytokines that lead to altered expression may result in variable susceptibility to ICD. In experimental skin irritation studies, polymorphism in the tumour necrosis factor-α (TNF-α) gene (TNFA-308) has been shown to play a role in the individual inflammatory response (5, 6). A pilot case–control study from our group, which compared 217 patients with occupational chronic ICD with 197 controls, did not find significant differences in genotype distribution for two TNFA polymorphisms in TNFA-308 and TNFA-238 (7). Only a subgroup analysis of different wet work exposures showed a trend of a higher prevalence of TNFA-308 variant genotype carriers (G/A and A/A) among patients with self-reported low levels of wet work than among those with a high level of wet work and the controls, indicating an increased susceptibility to ICD. Polymorphism in TNFA-238 showed a trend of a decreased prevalence of the variant allele in patients, but this was not significant. However, our pilot study included a limited number of subjects. To detect smaller effects of cytokine polymorphisms, larger cohorts were needed. In this article, we describe a reinvestigation of the distribution of TNFA-238 and TNFA-308 polymorphisms in relation to their prevalence in chronic ICD with greater numbers of patients and controls.

Methods

Study population

After approval by the ethics committee of the University of Osnabrück had been obtained, a cohort of 712 patients who were hospitalized at our clinic for treatment of occupational ICD of the hands were asked to participate in the study. Forty-seven patients chose not to enroll. After written informed consent had been obtained, a total of 665 patients were genotyped for two polymorphisms in the genes coding for TNF-α between November 2005 and March 2011. Patients were consecutively included in this study if they met the following inclusion criteria: (i) Caucasian, (ii) age ≥ 18 years, (iii) history of chronic ICD of the hands for at least 6 months, and (iv) no history of chronic inflammatory disorders (e.g. rheumatoid arthritis and psoriasis). One hundred and seventy-eight patients were excluded because of a primary diagnosis of allergic contact or atopic dermatitis, leaving a total of 478 patients with ICD for analysis. For each patient, a detailed medical history was taken, including information about sex, age, age of onset of the hand eczema, occupation, atopic diathesis, wet work exposure and final diagnosis. Patients were employed in one of the following occupational categories: (i) healthcare, (ii) metal work, (iii) hairdressing/beauticians, (iv) construction work, (v) food/catering, (vi) janitorial services, (vii) florist work and gardening, and (viii) others.

Controls were recruited from vocational schools training individuals for the same high-risk occupations as those of the patients (same source population). Of the 500 trainees asked to participate, 477 agreed. Controls provided written informed consent. Eighty-four of those were excluded because they were not Caucasians. The remaining 393 trainees were in their second or third year of schooling. The number of selected controls from each vocational training category was based on the patients per job category. The controls were asked to complete a questionnaire giving information about their sex, age, wet/irritant work exposure, and medical history, particularly with regard to the skin and to atopic diathesis.

For further analysis, patients were further classified according to their diagnosis regarding ICD with or without atopy. Atopy was diagnosed according to ongoing or past flexural eczema and/or if at least 10 points on the Erlangen atopy score were reached (8). For each patient, the duration of wet work was determined and classified as low (up to 2 hr/working day), moderate (2–4 hr/day), and severe (> 4 hr/day).

DNA sampling and isolation

DNA material was obtained from buccal mucosa cells with buccal swabs (Medispo, Oud-Beijerland, The Netherlands). For each subject, two swabs were obtained and placed in a 15 ml tube (Greiner Bio-one, Alphen a/d Rijn, The Netherlands) containing 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 with a commercial DNA isolation kit (Puregene®, Gentra Systems) based on a standard proteinase K digestion method, according to the manufacturer’s protocol. The tubes were stored at 4°C for up to 3 months prior to the DNA isolation. Upon DNA isolation, 12 μl of proteinase K solution (20 mg/ml; Merck, Darmstadt, Germany) was added to each tube and incubated overnight at 55°C. After cooling to room temperature, the swabs were placed in a 10 ml syringe (BD Plastipak, Franklin Lake, NJ, USA), positioned in a 50-ml tube, and centrifuged at 1500 g for 10 min. The solution obtained was added to the tube with the lysis buffer, and 0.7 ml of protein precipitation solution (Puregene®; Gentra Systems) was added. After centrifugation at 1000 g for 15 min, the supernatant containing the DNA was transferred to a new 15 ml tube.
and 2 ml of isopropanol (Fluka, Deisendorf, Germany) was added. The tube was then centrifuged at 1500 g for 15 min, and the supernatant was removed. The pellet was transferred to a 2 ml polypropylene tube (Fisher Emergo, Landsmeer, The Netherlands) and centrifuged at 9000 g for 10 min. After elimination of the supernatant, the pellet was washed with 1 ml of ethanol (70%) and again centrifuged at 9000 g for 1 min. Following the removal of the ethanol, the DNA pellet was resuspended in 100 μl of TE buffer (10 mM Tris and 1 mM EDTA; Sigma-Aldrich, Zwijndrecht, The Netherlands). The final amount of DNA was quantified by absorbance at 260 nm, and an aliquot was diluted to a working concentration of 2–10 ng/μl.

Cytokine genotyping
Genotyping of TNFA-238 (rs361525) and TNFA-308 (rs1800629) was performed by means of a fluorogenic 5′-nuclease polymerase chain reaction TaqMan® assay (Applied Biosystems, Foster City, CA, USA), with reagents obtained from Applied Biosystems (Primer/Probe Mix and TaqMan® Universal PCR Master Mix without AmpErase® UNG). Analyses were performed according to the manufacturer’s protocol on an ABI Prism 7700 sequence detection system (Applied Biosystems). Each plate contained two non-template controls and five allelic controls. Random replicate samples were determined (20% of all samples), and showed intrasubject concordance rates of >99%.

Statistics
The observed genotype frequencies were compared with the expected Hardy–Weinberg distribution by chi-square test. To estimate the risk of disease conferred by a particular genotype, we calculated the odds ratios (ORs) of being a patient with 95% confidence intervals (CIs). Comparing the heterozygous and homozygous variant allele genotypes with the homozygous wild-type genotype by use of the additive model, ORs and 95% CIs for allele frequencies were calculated with the chi-squared test, with the more frequent allele as reference. Patients or controls with incidental missing data were excluded from the statistical analysis of that specific variable. The statistical analyses were performed with SPSS™ version 16.0 (SPSS, Chicago, IL, USA).

Results
The general epidemiological characteristics of the patient population and the controls are shown in Table 1. Table 2 shows the number of patients according to different job categories, years of exposure to irritants at the time of ICD onset in patients, and subdiagnoses.

| Table 1. General epidemiological data of the study population and the controls |
|---------------------------------|-----------------|
|                                  | Patients        | Controls       |
| Proportion of females (%)        | n = 478         | n = 393        |
| Median age (years) (range)       | 60.3            | 61.0           |
| Median age at onset of hand eczema (years) (range) | 43 (18–67) | 19 (16–56) |
| n.a., not available.             |                 |                |

Genotype distributions
To avoid a selection bias of the controls, we compared the genotype distribution of our trainees with that of healthy German Caucasian blood donors (9). Both groups had similar allele frequencies (data not shown). In the patients and the controls, the genotype distributions of the single-nucleotide polymorphisms did not deviate from the Hardy–Weinberg equilibrium. Significant differences in genotype distribution were found when patients and controls were compared for the cytokine single-nucleotide polymorphisms TNFA-238 and TNFA-308. For a carrier of a variant TNFA-238A allele, the OR for acquiring ICD was 0.57 (95% CI 0.34–0.97), suggesting a protective effect of the A allele. In contrast, for a carrier of a variant TNFA-308A allele, the OR for acquiring ICD was 1.33 (95% CI 1.02–1.74), suggesting a detrimental effect of the A allele.

For detailed description of genotype and allele frequencies, see Table 3.

An additional subinvestigation explored possible associations of the two diagnostic groups (ICD with and without atopy) and genotype distributions with regard to polymorphisms. Genotype distributions in the two subgroups did not differ significantly from that in the controls (data not shown).

Cytokine single-nucleotide polymorphisms and flexural eczema
We investigated the possible association of cytokine single-nucleotide polymorphisms with ongoing or past flexural eczema. Flexural eczema was reported by 42.5% (n = 203) of our patients and by 20.6% (n = 81) of the controls. There was no association (chi-square test) between flexural eczema and any cytokine polymorphisms in either patients or controls (data not shown).
**Table 2.** Number of patients according to different job categories, years of exposure to irritants at the time of irritant contact dermatitis (ICD) onset, and subdiagnoses

<table>
<thead>
<tr>
<th>Job categories</th>
<th>Frequency, n (%)</th>
<th>Years of exposure at the onset of hand dermatitis, median (range)</th>
<th>Subdiagnoses, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthcare</td>
<td>188 (39.3)</td>
<td>14 (1–41)</td>
<td>ICD without atopy</td>
</tr>
<tr>
<td>Metal</td>
<td>101 (21.1)</td>
<td>20 (2–53)</td>
<td>137 (72.9)</td>
</tr>
<tr>
<td>Hairdressing, beauticians</td>
<td>59 (12.3)</td>
<td>7 (1–50)</td>
<td>52 (52.5)</td>
</tr>
<tr>
<td>Construction</td>
<td>28 (5.9)</td>
<td>24.0 (3–40)</td>
<td>48 (47.5)</td>
</tr>
<tr>
<td>Food and catering</td>
<td>35 (7.3)</td>
<td>12 (2–41)</td>
<td>45 (76.3)</td>
</tr>
<tr>
<td>Janitorial services</td>
<td>18 (3.8)</td>
<td>7 (1–28)</td>
<td>12 (34.3)</td>
</tr>
<tr>
<td>Florists and gardeners</td>
<td>10 (2.1)</td>
<td>24.5 (10–39)</td>
<td>23 (65.7)</td>
</tr>
<tr>
<td>Others/missing</td>
<td>39 (8.2)</td>
<td>—</td>
<td>14 (77.8)</td>
</tr>
<tr>
<td>Total</td>
<td>478 (100)</td>
<td>—</td>
<td>4 (22.2)</td>
</tr>
</tbody>
</table>

**Table 3.** Distribution of cytokine single-nucleotide polymorphisms (SNPs), genotypes and allele frequencies in irritant contact dermatitis patients (n = 478) and controls (n = 393)

<table>
<thead>
<tr>
<th>SNPs, genotypes, and alleles</th>
<th>Patients (n) (%)</th>
<th>Controls (n) (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α-238</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>437</td>
<td>331</td>
<td>0.64 (0.38–1.09)</td>
</tr>
<tr>
<td>G/A</td>
<td>26</td>
<td>31</td>
<td>0.6</td>
</tr>
<tr>
<td>A/A</td>
<td>0</td>
<td>2</td>
<td>n.a.</td>
</tr>
<tr>
<td>G allele</td>
<td>900</td>
<td>693</td>
<td>1.0</td>
</tr>
<tr>
<td>A allele</td>
<td>26</td>
<td>35</td>
<td>0.57 (0.34–0.97)</td>
</tr>
<tr>
<td>TNF-α-308</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>302</td>
<td>275</td>
<td>1.41 (1.03–1.90)</td>
</tr>
<tr>
<td>G/A</td>
<td>142</td>
<td>92</td>
<td>1.43 (0.55–3.74)</td>
</tr>
<tr>
<td>A/A</td>
<td>11</td>
<td>7</td>
<td>0.57</td>
</tr>
<tr>
<td>G allele</td>
<td>746</td>
<td>642</td>
<td>1.0</td>
</tr>
<tr>
<td>A allele</td>
<td>164</td>
<td>106</td>
<td>1.33 (1.02–1.74)</td>
</tr>
</tbody>
</table>

CT, confidence interval; OR, odds ratio; n.a., not available.
Different reference values because of failure of some genotype polymerase chain reactions.

Bold: significant results.

**Cytokine single-nucleotide polymorphisms and wet work exposure**

We investigated the assumption that individuals with cytokine single-nucleotide polymorphisms may show an increased propensity to develop ICD after exposure to lower levels of wet work. For this, our population was classified into patients with low (up to 2 hr/day), moderate (2–4 hr/day) and high (>4 hr/day) wet work exposure. Using ordinal regression, our results did not indicate an increased frequency of the variant alleles TNF-α-238 or TNF-α-308 in the low wet work exposure group (data not shown).

**Discussion**

Piguet et al. investigated the role of cytokines in the cutaneous response to the application of irritants in mice. The production of TNF-α mRNA by keratinocytes in mouse skin was markedly increased in ICD. Moreover, this group showed that pretreatment with anti-TNF-α antibodies inhibited the irritant response (10). Thus, there is considerable evidence suggesting that release of cytokines in diseased skin may be fundamental in the development of inflammatory dermatoses (11, 12).

The degree of variation observed in response to skin irritants (5, 6) and subsequent susceptibility to ICD may be a result of variations in the production of inflammatory cytokines coded by altered genes. Our study investigated two polymorphisms in the promoter positions -238 and -308 in the gene coding for TNF-α in patients with chronic irritant contact dermatitis. We observed associations for both cytokine polymorphisms, suggesting a protective effect of the A allele in TNF-α-238 and a deleterious effect of the A allele in TNF-α-308.

TNF-α, a proinflammatory cytokine, plays a key role mediating ICD responses (10, 12, 13). It is stored in dermal mast cells, but, following stimulation, it may be produced by keratinocytes and Langerhans cells (11, 14). Damage to the keratinocyte by irritants releases interleukin (IL)-1 (15). IL-1 stimulates further release of IL-1 and other proinflammatory cytokines from neighbouring keratinocytes. TNF-α liberation from IL-1-stimulated keratinocytes and macrophages enhances major histocompatibility complex (MHC) expression and antigen presentation to lymphocytes. In addition to this direct chemotactic effect, IL-1 and TNF-α induce the expression of intercellular adhesion molecules (ICAMs) on the surfaces of endothelial cells and fibroblasts that are recognized by circulating leukocytes. Leukocytes in turn adhere to the ICAM-expressing cells at the site of injury, and may then become activated by local inflammatory cytokine concentrations (15). Finally, this may lead to histological modifications and the clinical expression of dermatitis.

Although associations of TNF-α-238 and TNF-α-308 polymorphisms with susceptibility to a variety of diseases...
have been reported, consistent results have not been obtained until now. For TNFA-238, wild-type (G/G) carriers were more common among patients with rheumatoid arthritis with increased rate of joint damage and disease progress (16) and among patients with malignant melanoma (17). On the other hand, associations between the A allele in the -238 region were found for systemic sclerosis (18), chronic hepatitis B and C (19, 20), and juvenile onset of psoriasis and psoriatic arthritis (21–25). Additionally, Nedoszytko et al. and Reich et al. described a decreased prevalence of TNFA-308A in patients with early-onset psoriasis (23, 24).

The genes encoding TNF-α are located within the MHC region on chromosome 6p21.3, which is a highly polymorphic region. TNFA itself contains a large number of polymorphisms (26), and those most extensively investigated are at positions -238 and -308 (26–29). In Caucasian populations, the G→A transitions in promoter positions -238 and -308 represent the most common exchanges (30, 31).

Wilson et al. showed that the TNF2 allele (A allele) at -308 is a much stronger transcriptional activator than the common allele TNF1 (G allele) (32). This group also showed that the A allele at -308 is part of an extended MHC haplotype HLA-A1-B8-DR3-DQ2 (33), which, again, is associated with high TNF-α production (34). Results suggest that the TNF2 allele may be a useful marker for increased production of TNF-α, and that the AA genotype is associated with increased production of TNF-α. This may account for the increased susceptibility to ICD observed in individuals with the G→A polymorphism at position -308 (5).

Focusing on ICD, Allen et al. studied a possible relationship with a TNFA-308 polymorphism (5). They tested 221 volunteers with sodium dodecyl sulfate and benzalkonium chloride, and divided the responders into high and low irritant threshold groups. In the low irritant threshold group for both irritants, there was an increased frequency of the A allele in the -238 region compared to the AA genotype (35). Results suggest that the TNF2 allele may be a useful marker for increased production of TNF-α, and that the AA genotype is associated with increased production of TNF-α. This may account for the increased susceptibility to ICD observed in individuals with the G→A polymorphism at position -308 (5).

Davis et al. examined the effects of a G→A transition at position -308 on TNFA on chronically damaged skin of the hands and of non-affected skin of the lateral upper arms of healthcare workers. The genotype was determined in 68 healthcare workers with chronic ICD of the hands, and an investigation was performed of their clinical response (erythema, dryness, and barrier integrity) to repeated application of hand hygiene and after a recovery phase provided by a rest period. An excess of erythema during and after exposure suggested that GG individuals recover from irritation more readily than AA individuals. Moreover, in a subset of patients, irritation tests with water and sodium lauryl sulfate were conducted on clinically unaffected skin of the lateral upper arm. Both irritants produced a higher degree of erythema on normal skin for AA/GA than for GG patients. Taking the findings together, their study confirmed previous reports on the increased susceptibility to skin irritation of subjects with the G→A transition at position -308 in the promoter region of the TNFA gene (6).

In conclusion, our data provide additional insights into the complex pathomechanisms of ICD. The results showed that individuals with TNFA-238 polymorphisms are less prone and those with TNFA-308 polymorphisms are more prone to develop ICD of the hands, suggesting a protective versus a detrimental effect of the A allele, respectively. However, the relevance of an TNFA-308 polymorphism in an occupational setting can be regarded as low, as the OR for acquiring ICD was relatively low. The protective polymorphism in TNFA-238 showed a higher OR, but it was less prevalent in the investigated cohort.
TNF-α POLYMORPHISMS IN CONTACT DERMATITIS ● LANDECK ET AL.

References

TNFα POLYMORPHISMS IN CONTACT DERMATITIS  •  LANDECK ET AL.
