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Role of TNF-alpha polymorphism in patients with nickel allergy: a marker of susceptibility to contact polysensitization

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Abstract. – OBJECTIVE: Nickel allergy is the most frequent contact allergy in the industrialized country. In allergic contact dermatitis after the presentation of haptenated peptides by resident or newly recruited skin cells, activated CD8+ T cells release IFN-γ and TNF-α, these cytokines are potent activator of keratinocytes. The role of specific cytokines in nickel allergy is not yet fully elucidated. The adenine nucleotide at position -308 in the promoter region of the TNFA gene is associated with an increased production of TNF-α, that is a potent activator of keratinocytes.

PATIENTS AND METHODS: To evaluate the expression of TNF-α polymorphism in patients with allergic contact dermatitis and in healthy people, 41 patients with allergic contact dermatitis to nickel and 40 healthy controls were enrolled. A total of 81 subjects (41 cases and 40 controls) underwent genotyping for the 308 genetic polymorphism in the TNFA gene.

RESULTS: The distribution of TNF genotypes TNF-α 308 G/A polymorphism in cases didn't differ significantly in the controls group. The genotype GA was present in the 75% of the patients with polysensitization. In one patient was observed the rare genotype A/A.

CONCLUSIONS: The carriage of the TNFA–308 A/A and GA genotype may act as a marker of enhanced susceptibility to contact polysensitization, indicating that TNF-α is a key regulator of the initiation of delayed-type hypersensitivity reactions, the polymorphism seems to be not enough for the development of nickel monosensitization.

Key Words:
Contact allergy, Nickel, TNF-α, Polymorphism.

Introduction

Nickel allergy is the most frequent contact allergy in the industrialized country[1]. A review of all the epidemiological surveys conducted from 1966 to 2007, in Europe and USA, revealed a prevalence of nickel allergy ranging from 2.5% (Germany, 1966) to 17.6% (Norway, 2007)[2]; the cause of the elevated percentage of sensitization is mainly the daily use of jewellery containing nickel. In allergic contact dermatitis (ACD) after the presentation of haptenated peptides by resident or newly recruited skin cells, activated CD8+ T cells release IFN-γ and TNF-α, these cytokines are potent activator of keratinocytes[3]. TNF-α, with other cytokines, induces maturation of DCs (dendritic cells) such as Langerhans cells, to immune stimulatory cells and their migration from the skin to draining lymph nodes[4,5]. The adenine nucleotide at position -308 in the promoter region of the TNF-α gene is associated with an increased production of TNF-α[6,7]. The genotypes G/A and A/A has been associated with an highest risk for sensitization to chemical compounds[8]. Therefore, we supposed that ACD to nickel could be associated with TNF-α polymorphism.

Patients and Methods

In a prospective study, patients with nickel allergic contact dermatitis were enrolled at the Allergy Department of Policlinico A. Gemelli Rome, Italy, while control subjects were selected from the Division of Clinical Nutrition and Nutrigenomics, of the University of Rome Tor Vergata, Italy.

To reduce the variability were enrolled only women between 18 and 40 years.

DNA genotyping was performed at the Division of Clinical Nutrition and Nutrigenomics, University of Rome "Tor Vergata".

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Patients with severe chronic diseases, including active or previous cancers and with gastrointestinal or autoimmune diseases were excluded.

Diagnosis of allergic contact dermatitis was performed on the basis of the positivity of patch tests (European Standard Series).

Body Mass Index (BMI) was calculated using the formula: BMI = body weight (kg)/height (m^2) and classified according to the WHO BMI categories: underweight < 18.5 kg/m^2, normal weight 18.5-24.9 kg/m^2, preobese 25.0-29.9 kg/m^2, obese ≥ 30 kg/m^2.

Patch tests, European Standard Series, were applied on the interscapular region, checked at D3, in accordance with the European Environmental and Contact Dermatitis Research Group, and were considered positive if an eczematous-vesicular reaction occurred at the contact site with the allergen; the intensity was assessed with the following criteria: (1) erythema (±); (2) erythema, edema (+); (3) erythema, edema, vesicles, papules (++) (4) intense erythema, edema, confluent vesicles (+++).

TNF-α Genotype analysis

Genomic DNA was extracted from saliva using the QIAamp DNA Mini Kit (Quiagen, Valencia, CA, USA). TNF-α was selected for genotyping.

Sequencing of the 5'-region (-595 to +390) of the human TNF-α gene revealed a G to a A transition polymorphism at position -308. Digestion with NcoI enzyme, revealed the presence of two alleles: allele A1 gives two fragment of 87 bp and 20 bp, allele 2 a single 107 bp fragment. Amplification of 100 ng of genomic DNA was performed using initial denaturation at 94°C for 4 min followed by 30 cycles of 95°C for 1 min, 60° C for 1 min, and 72° C for 1 min with a final extension at 72° C for 10 min. The buffer for PCR reaction contained 2.5 mM $MgCl_2$, 9.9 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.1% Triton X 100, 0.200 µM deoxyribonucleotide triphosphate (dNTPs), 1 U of *Taq* DNA polymerase, and, 0.2 µM of each primers. The primer sequences are: 5'-AGGCAATAGGTTTTGAGGGCCAT-3', and 5' TCCTCCCTGCTCCGATTCCG-3'

The amplified product was digested with NcoI and analyzed on agarose gel, and visualized under UV light after ethidium bromide staining.

Statistical Analysis

Data were analyzed to check assumptions about the distribution of the measured variables. A χ^2-test was also used to evaluate the Hardy-Weinberg equilibrium of the observed genotype frequencies respect to the general population.

Comparisons among genotype groups were performed using an independent *t*-test, a non-parametric Mann-Whitney test. Odds ratios and 95% confidence interval were calculated for the risk assessment of nickel allergic contact dermatitis. All tests were considered significant for a *p*-value equal or less than 0.05 ($p \leq 0.05$). Statistical analysis were performed using a computer software package (SPSS for Windows, version 13.0; SPSS, Chicago, IL, USA).

Results

41 patients (female) with allergic contact dermatitis to nickel and 40 healthy controls (female), age between 19-40 (mean age 31 years) were enrolled.

4 patients had other contact sensitization (thimerosal, palladium, cobalt, chrome).

According to BMI classification, 6 subjects were underweight (5 mid-underweight BMI 17-18.49 kg/m^2 and 1 severe underweight BMI < 16.00 kg/m^2), 65 were in the normal range (BMI 18.50-24.99 kg/m^2), 7 were overweight (BMI 25.00-29.99 kg/m^2) and 1 was obese (BMI > 30 kg/m^2) (Table I).

Table I. Association between TNF-α -308 GG, GA, and AA genotypes and Nickel sensitized cases and controls.

Individuals	Total N	G/G n (%)	G/A +A/A n (%)	*p*
Controls	40	30 (75%)	10 (25%)	$p > 0.05$
Cases	41	35 (85%)	6 (14%)	$p > 0.05$
ACD Ni		34	3	
ACD Ni + other allergenes		1	3	$p \leq 0.05$

Legend: ACD = allergic contact dermatitis; Ni = Nickel.

All subjects underwent genotyping of the TNF-α -308 polymorphism. The TNF-α G/G homozygous genotype was observed in the 85% of cases and in the 75% of the controls. The TNF-α G/A polymorphism was in the 12% of cases and in the 25% of controls.

The distribution of G/A and A/A polymorphism between patients and controls did not differ significantly ($p > 0.05$).

Only one patient was A/A homozygote, and it was a polysensitized patient (nickel, cobalt, chrome) (OR 0.54, 95% CI 0.16-1.5).

By the way the 75% of polysensitized patients had the TNF-α G/A genotype, the magnitude of association between TNF-α A carriers (G/A and A/A genotypes) and individuals sensitized to contact allergen was increased restricting the analysis to females polysensitized (Table I). We found a significant association ($p \leq 0.007$) between G/A and A/A genotypes and multiple contact allergy.

Any significant association was found with the other variables (age, BMI, weight) evaluated.

Discussion

TNF-α, with other cytokines, induces maturation of DCs, such as Langerhans cells, to immune stimulatory cells and their migration from the skin to draining[4,5]. The pro-inflammatory cytokines, like TNF-α, are induced during the early phase of experimental contact hypersensitivity responses[9]. The transition from G to A has been identified at position -308 within the promoter region of the TNF-α gene, with the G form (allele 1) being the most common[10]. In literature there are studies reporting that the adenine nucleotide at position -308 in the promoter region of the TNF-α gene is associated with an increased production of TNF-α[6,7]. We therefore supposed that ACD to nickel could be associated with TNF-α polymorphism.

The allele frequencies observed in the controls in this study were comparable to data for mid-Europeans.

As reported in other studies the frequency of the allele A is rare.

Blomeke et al[8] observed the A/A genotype in 9/181 patients (5%) with sensitization to para-phenylendiamine (PPD) and we showed the same polymorphism in 1/41 patients with ACD. In accordance with published data, individuals with homozygous A/A genotypes were too rare for assessing statistical differences. The frequency of G/A genotype in patients with sensitization to para-phenylendiamine was 34% in cases and 21% in controls, in our patients with nickel allergic contact dermatitis frequencies of TNF-α G/A genotype were 12% and 25%. Blomeke et al[8] also observed that, the genotype G/A and A/A combined was significantly more common in the group of polysensitized patients (n = 86) (included individuals sensitized to PPD) compared with healthy controls, they concluded that females over 45 years, with G/A or A/A genotypes, have the highest risk for sensitization to PPD. In our study were included females under 40 years to minimize hormonal influences or age-related changes of the immune status. According to Blomeke et al[8], we showed that the genotype G/A was present in the 75% of the patients with polysensitization and that there is a significant association between the genotype G/A, A/A and the multiple contact allergy. However, this interpretation has to be confirmed, due to the limited number of polysensitized individuals included in the study. Davis et al[11] evidenced that TNF-α polymorphism and an atopic history affect the severity of irritation and recovery from exposure and response to treatment for common hand skin products in both chronic irritant hand dermatitis and normal skin. Therefore, we can assume about the role of TNF-α -308 G/A as a genetic component of both irritant and allergic contact dermatitis and it could reflect common pathogenetic pathways of these types of dermatitis.

In the study of Jensen et al[12], any differences in the serum levels of IL-2, IL-4, IFN-γ and TNF-α were seen before or after oral challenge with nickel in any of the groups studied (patients without cutaneous reaction after challenge, patients with cutaneous reaction after challenge and healthy controls).

This is the first study on the TNF-α polymorphism in patient with nickel contact allergy, since the occurrence of nickel allergy is higher in the general population compared to other allergic dermatitis to chemical compound, we can speculate that the low significantly statistic data are due to the small sample of patients. It should be addressed that the validity of this study is influenced by the composition of the study groups; nickel, differently from other chemical allergenes such as PPD, is world-wide distributed and the exposition to this metal is high in the general population and is age dependent.

Conclusions

According to previously published data, there are data supporting that the TNF-α -308 G/A polymorphism may influence susceptibility to ACD to chemical compound. From our data it seems that the TNF-α -308 A/A and G/A genotypes may act as a markers of enhanced susceptibility to contact polysensitization but the polymorphism seems to be not a risk factor for nickel monosensitization. More studies are needed to assess the correlation between TNF-α -308 G/A polymorphism and nickel allergy.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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