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CONTACT ALLERGY TO NICKEL: PATCH TEST SCORE CORRELATES WITH IL-5, BUT NOT WITH IFN-GAMMA NICKEL-SPECIFIC SECRETION BY PERIPHERAL BLOOD LYMPHOCYTES

Ewa Czarnobilska[1], Bartosz Jenner[1], Jolanta Kaszuba-Zwoinska[2], Maria Kapusta[3], Krystyna Obtułowicz[1], Piotr Thor[2], Radosław Śpiewak[4]

[1]Institute of Clinical and Environmental Toxicology, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[2]Institute of Pathophysiology, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[3]Department of Clinical Biochemistry, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[4]Institute of Dermatology Ltd., Kraków, Poland

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Abstract: Traditionally, allergic contact dermatitis (ACD) has been associated with the activity of Th1 lymphocytes that secrete interferon gamma. Recent evidence indicates that other cells, e.g. interleukin 5 (IL-5)-secreting Th2 or Tc2 cells may be among the key effectors of ACD. The aim of the present study was to assess the influence of nickel-specific IFN-gamma secretion (marker of Th1 and Tc1 activity) and IL-5 secretion (Th2 and Tc2) on the clinical outcome (patch test score) in nickel-allergic patients. 40 women with suspicion of ACD were involved, aged from 14–54 (median 31.5) years. They were patch tested with $NiSO_4$. Peripheral blood mononuclear cells (PBMC) from the patients were cultured and analysed for IFN-gamma and IL-5 secretion in response to $NiSO_4$. A series of statistical models (classical logit or cloglog link function) were used. We demonstrate that nickel-specific IL-5 secretion by PBMC is correlated with the intensity of patch test reaction (p=0.05), with no significant effect of IFN-gamma. An increase in the nickel-specific IL-5 secretion from PBMC by 10 pg/ml is associated with a 10–20% increase (depending on statistical model) in the odds ratio of the patient to have a higher patch test score. These findings support the assumption that cells secreting IL-5 (e.g. Th2, Tc2) play a more important role in the pathogenesis of ACD than previously thought.

Address for correspondence: Dr hab. med. Radosław Śpiewak, ul. Lentza 6/17, 31-312 Kraków, Poland. E-mail: spiewak@onet.eu

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INTRODUCTION

Allergic contact dermatitis (ACD) belongs to most frequent chronic diseases. Among Polish vocational students, lifetime prevalence of ACD was 10.9%, with point prevalence 1.6%, and patch test positivity 28.1% [24]. Comparable figures were observed among schoolchildren in Poland and Denmark [5, 15]. In Germany, the incidence of ACD is estimated at 1.8-7 new cases per 1,000 residents each year [21]. These examples illustrate the importance of ACD to public health. Despite the recognised importance and continuous efforts in the last 50 years, there is still no reliable method for *in vitro* diagnosis of ACD [25, 26]. One of the possible explanations for this could be that we still do not know the key phenomena involved in the pathophysiology of ACD.

Traditionally, ACD has been associated with the activity of Th1 lymphocytes [6, 11, 27], thus, most researchers

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Table 1. Results of studies on cytokine secretion in response to nickel from peripheral blood mononuclear cells – an overview of published data [27].

Cytokine	Interpretation	Difference between ACD and controls observed	No difference observed
IFN γ	"type 1"	[8, 10, 12, 14 20]	[2, 13, 26]
TNF α	"type 1"		[2]
IL-12	"type 1"		[10]
IL-2	both*	[3, 12, 26]	
IL-4	"type 2"	[2, 10, 12, 13, 14]	
IL-5	"type 2"	[2, 3, 8, 10, 14, 26]	
IL-13	"type 2"	[10, 13, 14, 26]	

*see explanation in the Discussion

focused on antigen-specific IFN-γ secretion. Recently, increasing evidence indicates that cells other than Th1 may be the key effectors of ACD [6, 7, 27]. However, the picture is still far from clear. Table 1 demonstrates that particular contradiction regards the secretion of IFN-γ by allergen-specific lymphocytes. The Table also collates evidence for the involvement in ACD of "type 2" cytokines, i.e. secreted by Th2 and Tc2 lymphocytes IL-4, IL-5 and IL-13. These cytokines have traditionally been associated with IgE-mediated, atopic allergy (Gell & Coombs Type I allergy – not to be confused with "type 1" cytokine IFN-γ, secreted by Th1 and Tc1) [22].

The most interesting results in this aspect were published by Minang *et al.*, who observed that the peripheral blood of persons with a higher patch test score to nickel contains both more lymphocytes secreting "type 1" cytokine IFN-γ and those secreting "type 2" cytokines IL-4, IL-5, IL-13 in response to nickel [14]. Unfortunately, the authors have not attempted to assess which particular cytokines determine the intensity of skin inflammation. In order to fill this gap, the present study was undertaken.

The aim of the study was to assess and compare the influence of nickel-specific IFN-γ and IL-5 secretion by peripheral blood mononuclear cells (PBMC) on the clinical outcome (patch test score) in nickel-allergic patients. Using advanced exploratory statistics, we have shown that nickel-specific IL-5 secretion determines the clinical outcome to a much higher extent than IFN-γ secretion. In fact, it appeared that the influence by Ni-specific IFN-γ secretion is negligible.

PATIENTS AND METHODS

40 women referred to the Allergy Department for routine patch testing due to suspected contact allergy to metals were invited to join the study. The participants were aged from 14–54 (median 31.5) years. The study protocol was accepted by the local Ethics Committee. After obtaining informed consent from the participant (or the legal guardian), blood samples were collected for peripheral blood mononuclear cells (PBMC) cultures. Subsequently, the patients were patch tested with $NiSO_4$ 5% in petrolatum (HAL Allergy) mounted in the IQ Chambers (Chemotechnique Diagnostics), along with other haptens selected according to the diagnostic needs. The scoring of patch test results was carried out according to the ICDRG rules [23].

Researchers responsible for cell cultures were not informed about the patch test result. From the collected blood samples, PBMC were isolated using density gradient (Ficoll Paque Plus, GE Healthcare) and immediately cultured in RPMI medium (Gibco BRL) supplemented with gentamicin-glutamine solution (Sigma Aldrich) and 10% human serum (Sigma Aldrich). PBMC from each patient were cultured in the density of 1×10^6 cells per well, both with and without the presence of $NiSO_4$ (final concentration 50 μM). Viability of the cells was confirmed by trypane blue exclusion and by unspecific activation with phytohemagglutinine (PHA). Concentrations of IFN-γ and IL-5 in culture supernatants were measured after 6 days by means of ELISA (Instant IFN-γ ELISA Kit, Hoelzel, and Quantikine IL-5 ELISA Kit, R&D Systems).

Statistical analysis. Relationships were analysed between cytokine secretion and scoring of patch test results on Day 3. The outcome measure from the clinical part was the patch test score, expressed on a ordinal scale ranging from "–" (no reaction) to "+++" (strong reaction) according to ICDRG [23]. The outcomes of laboratory tests taken for further analyses were the differences in cytokine secretion between cells cultured in the presence of nickel, minus the secretion of cells cultured without nickel (background activity). These differences, further referred to as "signal" were expressed in pg/ml. The data was analyzed by mean of logistic regression for ordered categories [1].

In the exploratory part of statistical analysis, we tested a series of models based on either classical logit or cloglog link function. Furthermore, we analysed the joint influence of independent variables (possible interactions) on the dependent variable, i.e. patch test score on Day 3. We also tried analyses with independent variables transformed in such a way that signals from IL-5 and IFN-γ were treated not only as a simple difference between the cytokine secretion in cell cultures with and without Ni, but also as a relative difference or logarithm of the difference. From the above-mentioned settings, we selected the 2 best models based on the AIC criterion [1]. In the more complicated model, 3 variables were taken into consideration: signal values of IL-5 and IFN-γ expressed as a simple difference, and age (categorized into "less than 45 years old" and "45 years or more"). The age was included into the analyses, as it is known to influence the risk for contact allergy [28]. In the second, simplified model, only the IL-5 signal was used as the explanatory variable, as in the first model it appeared to be the only relevant determinant. Both finally selected models were based on the logit function.

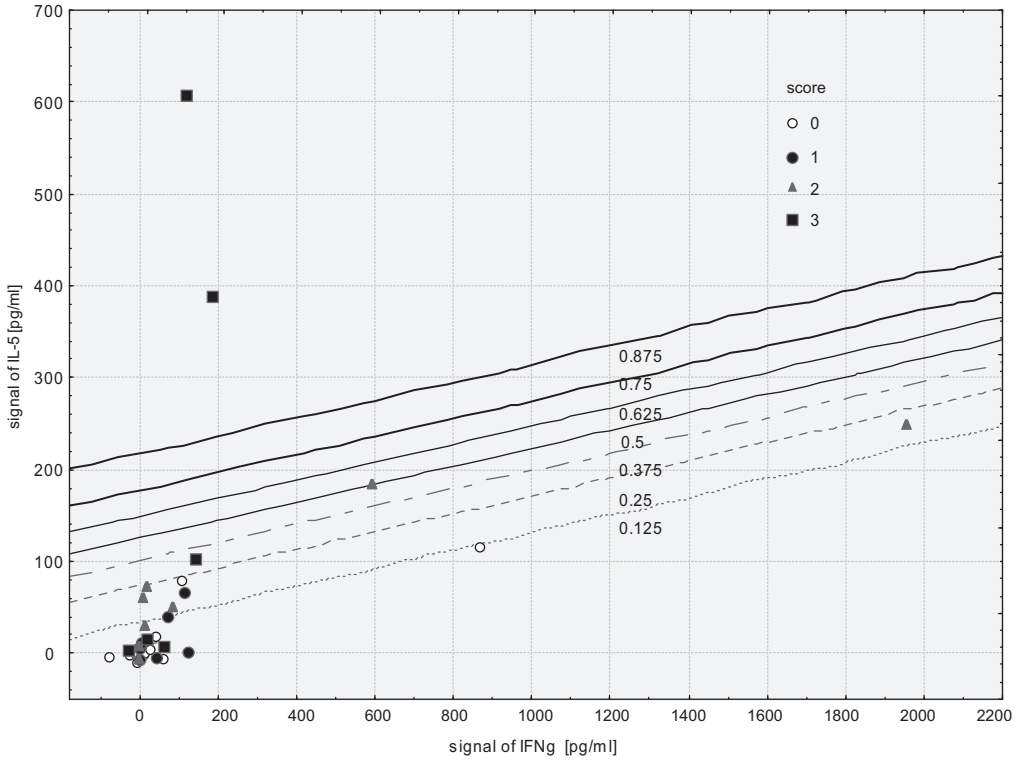

Figure 1. Original data and estimated isoclines of probability for a patient being patch test positive. Lines were calculated only for women below the age of 45 (majority of our patients). Lines for older patients would be slightly shifted toward the left upper corner.

RESULTS

Out of 40 women suspected of nickel allergy who entered the study, 3 were excluded because of either a doubtful (?+) reaction or outlier cytokine results: Of the remaining 37 persons, 19 were patch test negative to nickel on Day 3, 7 scored as +, 8 as ++, and 3 as +++. Patch test results were then correlated with nickel-specific IL-5 and IFN-γ secretion, and age. The results of the first model are shown in Table 2. The data suggests that an increase in the signal of IL-5 by 10 pg/ml is associated with a 24.2% increase in the predicted odds of a patient being in an upper category of score (p=0.05). In another words, the odds for being in the higher patch test score for a person with the signal x + 10 pg/ml are 1.24 times higher compared to person with the signal x pg/ml. Another tested parameter, i.e. categorized age and signal of IFN-γ, had no significant influence on the odds ratio. Figure 1 demonstrates how probability of contact allergy to nickel (in terms of positive patch test) depends on the intensity of signals from IL-5 and IFN-γ.

The output of the second model, where only the signal of IL-5 was analyzed, confirmed the usefulness of IL-5 secretion in predicting the patch test score. Using this model, we could still demonstrate that an increase in the IL-5 signal by 10 pg/ml is associated with an increase in the odds ratio of having a higher patch test score by 12.5% (95% CI: 2.6–23.5%, p=0.01), which shows similar trends as the results of the previous, more sophisticated model (24.2%). This relationship is illustrated in Figure 2.

Table 2. Parameters of logistic model for ordered categories. The model was designed to predict odds of being patch test positive. The fit of the model was measured as generalized coefficient of determination R2=0.33 [1].

parameter	df	estimate (95% CI)	SEE	OD (95% CI)	Wald χ^2	p
Intercept1	1	-2.73 (-4.05; -1.39)	0.68		16.06	< 0.01
Intercept2	1	-1.15 (-2.05; -0.24)	0.46		6.17	0.01
Intercept3	1	-0.20 (-1.02; 0.62)	0.42		0.23	0.63
signal of IFN	1	-0.02 (-0.05; 0.01)	0.02	0.98 (0.95; 1.01)	1.62	0.20
signal of IL5	**1**	**0.22 (0.00; 0.43)**	**0.11**	**1.24 (1.00; 1.54)**	**3.82**	**0.05**
Age ≥ 45	1	-1.29 (-3.51; 0.92)	1.13	0.27 (0.03; 2.52)	1.32	0.25

Df – degrees of freedom, SEE – standard error of estimate, OD – odds ratio, CI – confidence interval.

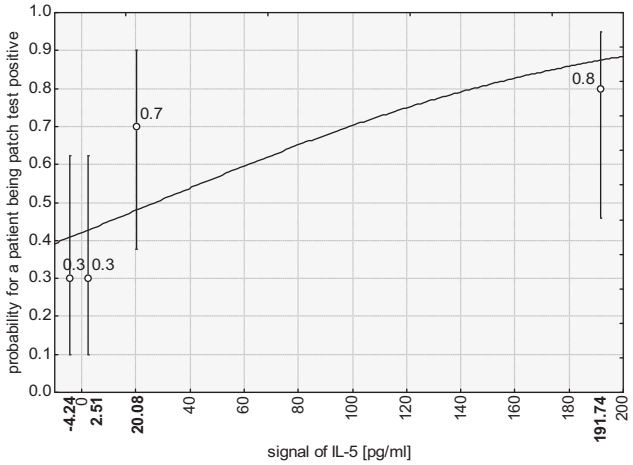

Figure 2. The relationship between intensity of signal of IL-5 and probability for a patient to be patch test positive. Observations were ranked by mean of intensity of IL-5 signal, and then divided into 4 groups of equal size (10 observations each). Within each group, empirical proportion of patch test-positive patients was calculated. These proportions are shown as dots surrounded with 95% CI (whiskers). Bold numbers at the horizontal axis denote arithmetic mean in each group. The superimposed line was calculated from the logistic model for ordered categories (generalized coefficient of determination R2=0.26).

DISCUSSION

We have demonstrated that the extent of nickel-specific IL-5 secretion – a marker of "type 2" lymphocyte activation – correlates with the intensity of patch test reaction. In contrast, the influence of "type 1" cytokine IFN-γ secretion and patient's age on patch test score were both negligible. Overall, our model shows that an increase in the Ni-specific IL-5 secretion in PBMC cultures by 10 pg/ml is associated with a 10–20% increase (depending on the statistical model used) in the odds ratio of the patient having a higher patch test score. This reinforces previous observations that nickel-specific secretion of "type 2" cytokines by PBMC may better discriminate between ACD patients allergic to nickel and controls (reviewed in Table 1). Our findings correspond well with the observations by Probst *et al.*, who have described the predominance of IL-5 secreting lymphocytes in lesional skin in ACD to nickel [18].

As shown in Table 1, nickel-specific IL-2 secretion also seems a good *in vitro* marker of contact allergy. Some authors consider IL-2 as a marker of "type 1" activation (Th1) [3, 19]. However, secretion of IL-2 is not merely restricted to the Th1 subtype. There is convincing evidence, that IL-2 is also secreted upon the first antigen encounter by naive T cells, yet before their phenotype (Th1 or Th2, Tc1 or Tc1) is determined [9]. Only after secretion of IL-2, naive CD4+ T cells mature into Th1 or Th2 phenotype, depending on whether they are exposed to IL-12 or IL-4 from their microenvironment [9, 17]. Similarly, CD8+ cells divide into cytotoxic lymphocytes Tc1 and Tc2 with secretion profiles similar to the respective T helper counterparts [4, 16]. Putting the above together, IL-2 should not be considered as a Th1 or Tc1 marker.

In summarising, the level of the allergen-specific IL-5 production by peripheral blood mononuclear cells appears as a relevant predictor of the patch test score, whereas IFN-gamma does not. This reinforces the assumption that "type 2" cells secreting IL-5 (e.g. Th2, Tc2, possibly also NK2 or NKT2) play an important (if not pivotal) role in the pathogenesis of allergic contact dermatitis. As most of the research carried out so far has focused on nickel allergy, further studies should explain whether this observation applies also to other haptens.

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