

ORIGINAL ARTICLE

IL-13: a marker of chromium contact allergy

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Abstract

Background Allergic contact dermatitis is a frequent, often disabling disease caused by countless substances. Patch testing remains the gold standard test to identify the causative agent; however, it is subjective, time-consuming and not completely safe. Alternative methods were tried, but significant success has only been achieved with nickel.

Objective Develop an alternative or complementary allergic contact dermatitis diagnostic test.

Methods We compared the lymphocyte proliferative rate and cytokine production (IFN- γ , IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17 and RANTES) between 18 chromium allergic patients and 19 controls.

Results The lymphocyte proliferation test and some of the cytokines tested (IFN- γ , IL-2, IL-5, IL-12 and IL-13) were able to discriminate allergic patients. However, striking results were only achieved using IL-13, leading to an accuracy of about 90%.

Conclusions If further studies confirm the data found, IL-13 could be used as an alternative or complementary test to detect chromium contact allergy whereas lymphocyte proliferation test, IFN- γ , IL-2, IL-5 and IL-12 detections may serve as additional diagnostic tests.

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Conflict of interest

None.

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Introduction

Allergic contact dermatitis (ACD) is a common and often disabling disease mediated by T cells against haptens.^{1–3} Chromium is the third frequent cause for metal sensitization. Although it is found in daily goods, sensitization is particularly relevant in the construction, tanning, pottery and metal industry.^{3,4} Despite patch testing (PT) is the gold standard to identify the causative allergen of ACD, it is time-consuming, subjective and not completely safe.^{5–8} Furthermore, it is contraindicated in active disease, pregnancy, recent sun exposure and the test reproducibility have shown some divergence in the same subjects.^{5–9} Another drawback is the high rate of irritant, false positive, reactions specially seen with metal testing.¹⁰ Thus, the development of a blood-sample-based diagnosis is of high interest. Lymphocyte proliferation testing (LPT) is an *in vitro* assay that evaluates lymphocyte antigen recall. LPT is objective, harmless and may be used when patch testing is contraindicated.^{6,11} Nevertheless, good results have only been achieved with nickel.^{12,13} Despite several methodologies tested, low sensitivity rates have been consistently found with

chromium LPT.^{3,14–18} In an attempt to optimize chromium LPT alternative forms of this method, such as (i) use of dendritic cells instead of monocytes, (ii) use of cytokines or mitogens to boost the reaction and (iii) detect the response by cytokine measure reopened the doors for further investigations.^{18–22} Nonetheless, the few studies that followed these protocols have considerable differences in their methodology and drawbacks questioning its clinical applicability. To define the role of cytokines in the detection of chromium LPT response we compared the traditional proliferation rate index with the measurement of various cytokine.

Methods

Patients and controls

Eighteen patients (17 males and 1 female with mean age of 50 years, range 36–65) with proven allergy to potassium dichromate by medical history, physical examination and patch testing were enrolled in this study as case group. Another 19 patients (11 males and 8 females with mean age of 44 years, range 26–68) with

ACD caused by other allergens, that is, without history of chromium allergy and with negative patch test to this metal, were used as control group. Twelve controls were sensitized to one or more allergens as denoted by clinical and patch testing findings. There were no significant age difference between the groups ($P = 0.1913$). Informed consent, approved by the local ethical committee, was obtained from all participants.

Patch testing

Patients and controls were patch tested using the Brazilian standard patch testing series (IPI-ASAC Brasil[®], Rio de Janeiro, Brazil), which comprises 30 antigens, including 0.5% potassium dichromate in petrolatum. The allergens were applied under Finn chambers for 2 days on sticky tape (3M, St Paul, MN, USA) over the upper back. Results were read at 48 and 96 h, and the reactions were scored following the ICDRG recommendation: + = erythema and infiltration, ++ = erythema, infiltration and vesicles and +++ = erythema, infiltration and confluent vesicles.⁵ Erythema and infiltration were the minimum criteria for considering a patch test positive.

Chromium lymphocyte proliferation assay

Mononuclear cells were isolated from heparinized venous blood by centrifugation on a density gradient (Ficoll-Hypaque, Piscataway, NJ, USA). They were washed and resuspended (2×10^6 cells/mL) in RPMI 1640 medium supplemented with gentamicin and 10% pooled normal human AB serum. Aliquots (100 μ L) of cell suspension were cultivated with either 100 μ L of RPMI 1640 medium (non-stimulated cultures) or 100 μ L of 666 μ g/mL chromium chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$; purity $\geq 96\%$; Merck, Darmstadt, Germany) solution prepared in RPMI 1640 medium (stimulated cultures), as previously described.⁴ Cells were also stimulated with 5 μ g/mL of pokeweed (*Phytolacca americana*; Sigma, St Louis, MO, USA) as positive controls. Cultures were performed in triplicate and duplicate in two separate 96-well flat-bottomed plates (TPP, Trasadingen, Switzerland) for 6 days. The triplicate cultures and duplicate cultures were used to determine the proliferation rate and the cytokine expression respectively.

Proliferation rate

Eighteen hours before cell harvesting, 1 μ Ci of radiolabelled thymidine (Amersham Pharmacia Biotech, Amersham, Buckinghamshire, UK) was added to each culture. T-cell proliferation was measured by ^3H -thymidine incorporation detected by a scintillation counter (Betaplate, Wallac, Finland). The stimulation index (SI), calculated as the ratio between the average ^3H -thymidine uptake found in the three stimulated and the three non-stimulated cultures of each patient, was used to determine the lymphoproliferative response ($\text{SI} = \text{average } ^3\text{H}\text{-thymidine count in three stimulated culture} / \text{average } ^3\text{H}\text{-thymidine count in three non-stimulated culture}$). Reactivity was considered positive if $\text{SI} > 3.0$.

Cytokine assay

Expression of 9 cytokines (IFN- γ , IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17 and RANTES) was measured in the culture's supernatants obtained from patients and controls. The supernatants were collected after 6 days of culture and frozen at -70°C . All samples were coded and the analysis ran blindly in an independent laboratory. The multibead array Milliplex Analyser v2.3[®] (Millipore Inc., Billerica, MA, USA) was used to quantify the cytokines, according to the manufacturer's instruction. The cytokine mathematic ratio (CR) and difference (CA) found between the stimulated and non-stimulated cultures were used to access the results.

Statistical analysis

The non-parametric Mann-Whitney U -test was used to compare non-stimulated and stimulated cultures, SI and cytokines measures between patients and controls. Correlations tests were calculated using Spearman's non-parametric correlation test. For the correlation tests, the variable allergy was graduated in 0, 1, 2 and 3 according to the patch test results (negative, +, ++ and +++ respectively). Significance was set at $P < 0.05$. The statistical analyses were calculated using Graph Pad Prism (GRAPHPAD software Prism[®] version 3.0; GraphPad Software, San Diego, CA, USA).

Results

Patch testing

All chromium allergic patients had positive patch test results to potassium dichromate, one (+), thirteen (++) and four (+++). Besides chromium, eight patients were sensitized to carba-mix, five to thiuram-mix and nickel sulphate, three to cobalt and one to PPD-mix, formaldehyde, quinoline and nitrofurazone.

Five subjects of the control group were sensitized to nickel sulphate, two to cobalt, colophony, neomycin, fragrance mix and one to benzocaine, chlorhexidine, ethylenediamine, formaldehyde, nitrofurazone, thimerosal and thiuram-mix.

Lymphocyte transformation test

Both groups showed adequate ($P < 0.0001$) and similar ($P = 0.2185$) proliferation when stimulated with pokeweed. However, only the allergic patients responded to chromium stimulation with increase in ^3H -thymidine uptake ($P < 0.0001$ and 0.2202) leading to different stimulation indexes between the groups, $P = 0.0014$. Six of the 18 cases and no controls had stimulation index above 3. The lymphocyte proliferation test sensitivity, specificity, accuracy (the proportion of correct predictions) and correlation with allergy to chromium were 33%, 100%, 68% and 0.56 respectively.

Luminex[®] cytokine assay

Chromium induced the production of IFN- γ , IL-5 and IL-13 in the case group, $P = 0.005$, 0.006 and <0.0001 , respectively, but not in the control group. This led to differences in both CR ($P = 0.0186$, 0.0032 and <0.0001) and CA ($P = 0.0186$, 0.0102

and <0.0001) between groups. The sensitivities, specificities, accuracies, cut-offs, and correlation values of these cytokines are detailed in Table 1.

There was no difference in IL-2 and IL-12 expression between stimulated and basal cultures in both groups. However, the production of IL-2 led to different CR ($P = 0.0465$), but not CΔ ($P = 0.0705$) and the IL-12 different CR ($P = 0.0024$) and CΔ ($P = 0.0050$) between groups. The results of these diagnostic indexes are available in Table 1.

Both groups showed an increased expression of RANTES ($P < 0.0001$), when stimulated with chromium resulting in similar diagnostic indexes (CR $P = 0.5535$ and CΔ $P = 0.6595$). On the other hand, chromium was not able to alter the basal expression of IL-4 and IL-17 in cases and controls making the diagnostic indexes useless. With regard IL-10, the stimulus decreased the expression of this cytokine in volunteers with and without allergy generating non-significant indexes.

Discussion

LPT is not sufficient sensitive enough to replace PT for chromium allergy diagnosis.³ A few alternative protocols were tried and their results encourage further investigation.^{18–22} This study compared the response of chromium allergic patients and controls with conventional LPT and various cytokine expression without any boost in both tests. The cytokines used were chosen by their involvement in the immunopathogenesis of ACD or by a shift on their expression on previous studies.^{3,19–22}

The response to 'pokeweed' was adequate and showed equal intensity in cases and controls, confirming that the culture conditions were suitable and that cases and controls respond similarly to stimuli that both are sensitive. On the other hand, only allergic patients' lymphocytes were stimulated with chromium. However, the sensitivity was too low confirming that low sensitivity is a feature of this test. A moderate correlation between allergy and SI values was found in this and Lindemann *et al.* study.²²

Chromium induced the expression of IFN- γ , IL-5 and IL-13 only in the case group. The sensitivity and specificity found with IFN- γ were similar to those obtained by Trattner *et al.* (73.7% and 71.4%) and Moed *et al.* (57% and 86%), but they used the mitogen

phytohemagglutinin and cytokines, respectively, as additional stimulus to increase the reaction and thus the test sensitivity.^{19,20} At a glance, we could question the efficacy of those boosts once the results were comparable. However, the incubation period of Trattner *et al.* was considerably shorter and the cohorts studied by Moed *et al.* were too small, limiting the comparison.^{19,20} Lindemann *et al.* also found an increase in IFN- γ expression in allergic patients, but they did not calculate the sensitivity and specificity of the method.²² Only Minang *et al.* found no difference in the production of IFN- γ , perhaps due to the small number of patients tested.²¹

The IL-5 production is increased in chromium allergic patients' stimulated cultures as demonstrated by Moed *et al.* and us.²⁰ Moed *et al.* obtained spectacular sensitivity and specificity ratios, 100% and 86% respectively. Nonetheless, the use of cytokines to boost the response and the small number of patients tested may be responsible for those outstanding numbers. Although the accuracies found with IFN- γ and IL-5 in this study do not allow the routine replacement of PT, they may be used as an alternative or additional tool.

The IL-13 accuracy was amazing. Minang *et al.* found an increase in IL-13 in five of nine chromium allergic patients.²¹ Three of the patients that have not responded had only erythema and oedema on patch testing and at least one strong positivity to another metal. Besides the possibility of interference by the positivity of other substances, the difficulty in separating true allergic from irritant reactions and the poor reproducibility of weak reactions with PT lead to the idea that the sensitivity of the test fell short of its possibility because questionable cases were included. Another explanation is that weak patch test reactors are not well detected by IL-13 quantification. In our cohort, the only patient who had a weak positive reaction with patch test responded to both IL-13 diagnostic indexes.

Because up to one in two positive reactions to 0.5% potassium dichromate in the patch test is false-positive, testing with 0.25% is recommended in the United States. However this is not universally accepted as there is a decline in sensitivity. Considering the excellent results encountered with IL-13 detection and the pitfalls of chromium patch testing, we can assume that IL-13 quantification is able to replace chromium path test if additional studies confirm

Table 1 Sensitivities, specificities, accuracies, cut-offs and correlation values found with assays that were able to discern chromium allergic patients from controls

	LPT	IFN- γ		IL-2	IL-5		IL-12		IL-13	
	SI	CR	CΔ	CR	CR	CΔ	CR	CΔ	CR	CΔ
Cut-off	>3	>6	>50	>1.2	>2	>0.2	>1.5	>0.3	>3	>2
Sensitivity	33%	83%	72%	61%	72%	56%	50%	60%	89%	94%
Specificity	100%	63%	68%	84%	79%	89%	84%	74%	89%	89%
Accuracy	68%	73%	70%	73%	76%	73%	68%	70%	89%	92%
rs	0.55	0.40	0.42	0.38	0.49	0.49	0.42	0.38	0.82	0.82

CΔ, cytokine difference; CR, cytokine ratio; LPT, lymphocyte proliferation test; rs, Spearman's correlation test found between SI, CR or CΔ and chromium allergy; SI, stimulation index.

these data. The detection of IL-13 also looks promising as a diagnostic method for other antigens and to distinguish allergic from irritant contact dermatitis.^{23–25} Despite these excellent results, the role of IL-13 in the immunopathogenesis of ACD remains unclear, in recent reviews, this cytokine is not even mentioned.^{1,2}

Although IL-2 CR, IL-12 CR and IL-12 CA were able to discern allergic patients from controls, the low accuracy found with them limit their clinical applicability. Both groups showed a similar increase in RANTES and decrease in IL-10 expressions resulting in useless diagnostic indexes. Chromium was not able to alter the basal expression of IL-4 and IL-17 in cases and controls making the diagnostic indexes also worthless.

We demonstrated that the detection of IL-13 may be able to replace PT and that INF- γ and IL-5 are suitable as alternative or additional testing. The importance of the subject and the results so far obtained encourage further research to confirm these results, extend them to other antigens and refine the method.

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