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Poster Presentation
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In vitro T Cell Reactivity in Nickel Allergy: Comparison of T Cell Clonality, Cytokine Expression and Mediator Production

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Key Words
Lymphocytes · Nickel allergy · T cell receptor · IL-4 · IFN-γ · PCR

Introduction

Apart from typical symptoms of nickel contact allergy, there are also unusual manifestations, e.g. pseudolymphoma formation, airborne contact dermatitis or implant-associated intolerance reactions [1]. Delayed-type hypersensitivity to nickel is reflected by eczematous reactions upon patch test and by an antigen-specific T lymphocyte proliferation in vitro [2–4]. We now assessed to what extent peripheral blood mononuclear cells (PBMC) from nickel-allergic and from nonallergic individuals would proliferate in response to nickel stimulation in vitro. In addition, the production of IL-4 and IFN-γ in these cultures was monitored by RT-PCR of the respective mRNA and by immunoassay of the supernatants. In addition, we examined with a PCR analysis of the several families of the T cell receptor gamma (TCR-γ) gene, if instead of being randomly stimulated T cells would show a clonal expansion pattern.

Materials and Methods

Suspensions of PBMC were obtained by centrifugation from the peripheral blood of 10 nickel-allergic individuals and 5 controls not allergic to nickel. The subsequent cell culture was performed either with complete 10% AB serum containing medium alone or with the addition of phytohemagglutinin (PHA; 2.4 μg/ml), tetanus toxoid (TT; 5.0 μg/ml) or nickel sulfate (10^{-4} *M*, 10^{-5} *M*). On day 5 a proliferative response was assessed by ^{3}H-thymidine uptake and expressed as stimulation index.

Released IL-4 [detection limit (d.l.): 1.5 pg/ml] and IFN-γ (d.l.: 5.0 pg/ml) in the supernatants were measured by ELISA. Samples below d.l. were set to 0.75 pg/ml (IL-4) and 2.5 pg/ml (IFN-γ). To evaluate the respective actual mRNA levels extraction by the phenol/chloroform method was done. RT-PCR (IL-4, IFN-γ, β-actin) was applied according to standard protocols. To cover the range of the TCR-γ chain we used a combination of primers for the main groups of the variable region (Vγ1–8, Vγ9, Vγ10, Vγ11) and a primer mix, which contains several sequences of different joining regions. The TCR region Vγ1–8 was detected by the consensus primer Vγ2. PCR products were separated on a 6% polyacrylamide gel and photographed after staining with ethidium bromide. Clonal expansion of few specifically reacting T cell populations would result in defined bands in contrast to a ‘smear’ in the case of polyclonal TCR-γ rearrangement.

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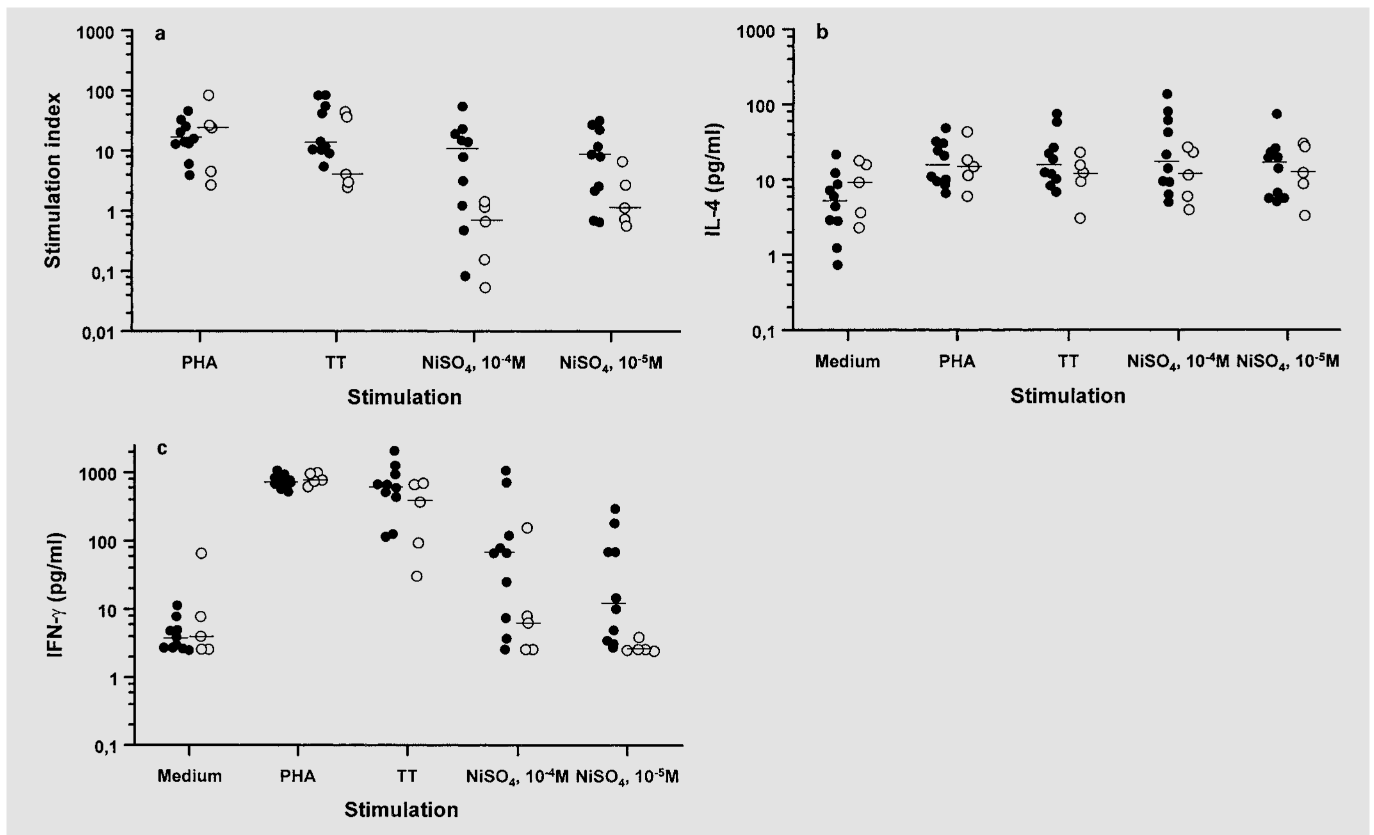

Fig. 1. a Proliferative response (stimulation index) from PBMC of 10 nickel-allergic and 5 nonallergic individuals. **b** Production of IL-4 from PBMC of 10 nickel-allergic and 5 nonallergic individuals. d.l. was 1.5 pg/ml (samples below d.l. are expressed as 0.75 pg/ml. **c** Production of IFN-γ from PBMC of 10 nickel-allergic and 5 nonallergic individuals. d.l. was 5.0 pg/ml (samples below d.l. are expressed as 2.5 pg/ml). ● = Nickel-allergic; ○ = nonallergic.

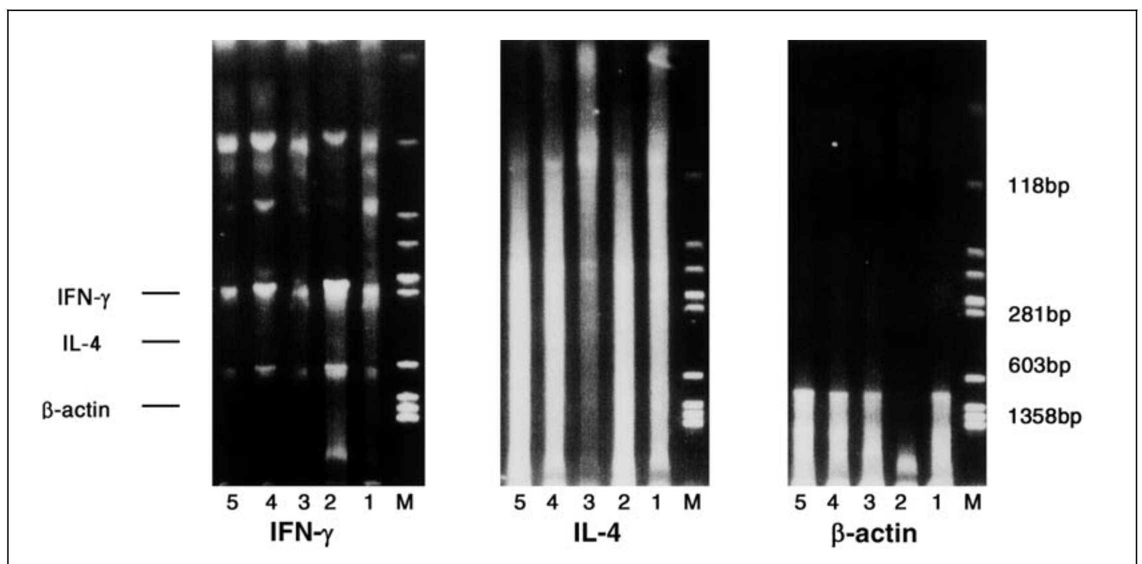

Fig. 2. RT-PCR products of mRNA from cell culture series (here nickel-allergic individual No. 10). 1 = Medium; 2 = PHA; 3 = TT; 4 = $NiSO_4$ 10^{-4} *M*; 5 = $NiSO_4$ 10^{-5} *M*; M = DNA marker.

Table 1. Analysis of TCR-γ specificity upon medium or allergen stimulation (clonality)

Allergic individuals	Medium alone	PHA	TT	$NiSO_4$ 10^{-4} *M*	$NiSO_4$ 10^{-5} *M*
1	–	–	Vγ11	–	–
2	–	–	Vγ11	Vγ10	–
3	–	–	Vγ11	Vγ10	Vγ10
4	–	–	Vγ9	Vγ9	Vγ9
5	–	–	Vγ11	Vγ2, Vγ11	–
6	Vγ9	Vγ9	Vγ9	Vγ9	Vγ9
7	–	–	–	Vγ10	–
8	–	–	Vγ11	Vγ10	Vγ10
9	–	–	–	–	–
10	–	–	Vγ9	Vγ2	Vγ2
Controls					
11	–	–	Vγ11	–	Vγ11
12	–	–	Vγ9	Vγ2, Vγ9	Vγ9
13	–	–	Vγ9	–	–
14	Vγ10	Vγ2	Vγ10	Vγ2	–
15	–	–	–	Vγ11	Vγ10

– = No preferential TCR-γ expression; Vγ2, Vγ9, Vγ10, Vγ11 = predominant TCR-γ family.

Results and Discussion

PBMC of nickel-allergic patients proliferated upon the addition of nickel sulfate to a varying degree. Cells of the different blood donors – some of whom had actual eczema – showed a baseline IL-4 production. In contrast, IFN-γ in the supernatants was enhanced in nickel-stimulated cultures of allergic individuals (fig. 1). This was reflected by the predominant detection of IFN-γ-specific mRNA on day 5 (tables 1, 2, fig. 2). Despite most T cells showing surface expression of TCR-αβ, the analysis of the concomitant rearrangement of TCR-γ gene makes it possible to evaluate the array of T lymphocytes with regard to clonality. Here the analysis of the TCR-γ spectrum in the specifically stimulated cultures showed clonal expansion (tables 1, 2) in contrast to the unspecific, broad stimulation by PHA. This would point to a rather specific response in sensitized individuals in contrast to random T cell activation. Thus, the combined analysis of proliferative response in vitro, mRNA characterization and potential clonal TCR-γ rearrangement [5, 6] could further help to identify specific antigen/allergen-induced T cell activation.

Table 2. Qualitative evaluation (band intensity) of RT-PCR products (IL-4/IFN-γ)

Allergic individuals	Medium alone		PHA		TT		$NiSO_4$ 10^{-4} *M*		$NiSO_4$ 10^{-5} *M*	
	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ	IL-4
1	–	–	+++	–	++	–	++	–	+++	–
2	–	–	+++	++	++	–	+++	–	++	+
3	–	–	+++	+	+	–	+++	–	+++	–
4	–	–	+++	–	–	–	++	–	+++	–
5	–	–	+++	–	+++	–	+++	–	+++	–
6	+	–	+++	–	++	–	+++	–	+++	+
7	–	–	+++	–	++	–	+++	–	+++	–
8	+	–	+++	+	+	–	++	–	++	–
9	+	–	+++	–	++	+	+++	–	+	–
10	++	–	+++	–	+	–	+	–	++	–
Controls										
11	–	–	+++	+	–	–	+	–	–	–
12	–	–	+++	–	+	–	+	–	+	–
13	–	–	+++	+	++	–	–	–	+	–
14	–	–	+++	–	–	–	+	–	–	–
15	–	–	+++	–	–	–	–	–	+	–

mRNA was extracted from unstimulated (medium alone) or allergen stimulated cultures. – = Absent; + = visible band; ++ = strong band; +++ = very strong expression. Evaluation was done with reference to β-actin expression.

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