

Impaired hapten sensitization in patients with autoimmune disease

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Introduction

Interestingly, recent epidemiological studies have shown that an inverse relation exists between contact allergy and the autoimmune diseases: psoriasis, diabetes type I, rheumatoid arthritis and inflammatory bowel disease [1–4]. Two experimental sensitization studies have shown reduced reactivity to challenge in patients with psoriasis [5,6], but the ability to become sensitized was not investigated. One study has found a reduced sensitization ratio among patients with rheumatoid arthritis [7], but the sensitization ratio and reactivity of patients with other autoimmune diseases have not been investigated and the mechanisms behind the apparent impairment in contact allergy remain unknown.

Contact allergy is highly regulated, due in part to regulatory T cells playing a role in diminishing collateral damage and helping in the resolution of allergic contact dermatitis (ACD). Regulatory T cells may even help in preventing ACD altogether, indicated by recent studies showing that in non-

Summary

An inverse relation between contact allergy and autoimmune diseases is suggested from epidemiological studies. The aim of this study was to investigate susceptibility and reactivity in patients with psoriasis, patients with diabetes and healthy controls in an experimental sensitization study. We sensitized 68 adult individuals (23 patients with psoriasis, 22 patients with diabetes and 23 healthy controls) with diphenylcyclopropenone (DPCP) and assessed challenge responses with visual scoring and ultrasound. Skin biopsies from challenged skin were investigated for differences in down-regulatory mechanisms with immunohistochemistry and gene-expression profiles using microarray technology. The sensitization ratios were 26%, 36% and 65% for the psoriatic, diabetic and healthy groups, respectively. Logistic regression analysis gave an odds ratio (OR) for a patient with psoriasis or diabetes type I of being sensitized to 0.18 [95% confidence interval (CI): 0.039–0.85], $P = 0.031$ and 0.74 (95% CI: 0.548–1.008), $P = 0.056$, respectively. A high degree of forkhead box P3-positive (FoxP3⁺) cells were found in biopsies of positively challenged reactions, but only limited numbers in negatively challenged reactions, with no difference among the groups. No specific mRNA expression was found in the challenged skin of negative elicitation reactions, also indicating no sign of active down-regulation. The study contributes strongly to the evidence of a decreased susceptibility to develop contact allergy in individuals with autoimmune diseases such as psoriasis.

Keywords: autoimmune disease, contact allergy, psoriasis, sensitization

allergic individuals antigen-specific regulatory T cells are activated and found in the challenge sites and blood of non-allergic individuals [8,9]; thus, an active down-regulation is taking place.

The aim of our study was, first, to investigate in a controlled human sensitization study the ability of becoming sensitized among patients with two different autoimmune diseases, psoriasis and diabetes type I, and secondly to identify whether or not down-regulatory events were present in the elicitation phase by investigating skin biopsies taken from elicitation sites with immunohistochemistry and mRNA expression profiles with microarray analysis.

Materials and methods

Subjects

Sixty-eight adult patients were included in the study: 23 patients with psoriasis (13 women, 10 men, mean age 50.7

years), 22 patients with diabetes type I (10 women, 12 men, mean age 40.0 years) and 23 healthy controls (14 women, nine men, mean age 34.6 years).

Patients with psoriasis were recruited from the Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark. Patients with diabetes were recruited from Steno Diabetes Centre, Gentofte, Denmark and healthy controls via advertisement.

Inclusion criteria were: (i) age between 18 and 65 years of age; and (ii) for psoriasis patients, a diagnosis of psoriasis verified clinically by a specialist in dermatology and for diabetes patients a diagnosis of type I insulin-dependent diabetes. Exclusion criteria were: (i) women who were either pregnant or breast feeding; (ii) use of corticosteroids or other immunosuppressive drugs; (iii) exposure to sunlight for 2 weeks; (iv) a history of atopy; (v) previous exposure to diphenylcyclopropenone (DPCP); and (vi) current malignancy or a history of malignancy.

The study was approved by the local ethics committee (journal number H-C-2007-0123) and all subjects included gave written informed consent before enrolment.

Sensitization

All subjects were sensitized with DPCP in acetone on buttock skin. Petrolatum-backed 11-mm filter disks were soaked in 50 µl of 0.0625% DPCP in acetone (25 µg/50 µl). Each filter disc was mounted inside a 12-mm aluminium Finn chamber® and taped to the skin (Scanpor®; Epitest Oy, Tuusula, Finland). The disks were left for 48 h.

Challenges were carried out on the upper inner arm 3 weeks after sensitization, using four concentrations of DPCP in acetone (0.39, 0.78, 1.56, 3.125 µg/15 µl) and one acetone control on 7-mm filter discs in 8-mm Finn chambers®. The discs were left for 6 h. The challenge sites were all marked with a surgical skin marker for evaluation 48 h later.

Sensitization as well as challenge was performed on healthy skin.

Visual assessment

The elicitation responses were assessed using a visual score, as suggested by Cooper and co-workers [10]: 1 = no reaction, 2 = mild, macular erythema, 3 = moderate erythema, occasionally with papulation, 4 = strong erythematous reaction (including vesicular changes) and 5 = extreme or spreading reaction (including bullous or ulcerative reaction). The sum increase in clinical score was calculated as the sum of values at the five challenge sites.

Ultrasound assessment of skin thickness

Increase in dermal thickness, measured using the ultrasound technique, has been shown to correlate well with the strength

of an elicitation reaction [11]. In addition to visual scoring, dermal thickness of each elicitation site was determined using a high-frequency ultrasound scanner (Dermascan, Cortex Technology, Horsens, Denmark). Twelve-mm scanned images were recorded pre- and post-challenge. Five dermal thickness measurements were taken from each pre- and post-challenge scan at fixed distances of 2, 4, 6, 8 and 10 mm along the horizontal length of the scanned image. A mean percentage increase in dermal thickness was calculated for each elicitation site.

Biopsies

Two 4-mm punch biopsies were taken from each patient, one from the challenge area where the highest dose of DPCP (3.125 µg/15 µl) had been applied and one from the area where acetone had been applied. The biopsies were taken 48 h after challenge. Biopsies were taken from 29 individuals; 11 were used for immunohistochemistry and 17 were used for the microarray study.

Immunohistochemistry

Biopsies taken from 11 individuals, six patients with psoriasis, two of whom had a positive elicitation reaction and five healthy controls, three of whom had a positive elicitation reaction, were prepared for immunohistochemistry. These skin biopsies were embedded in Tissue Tek octreotide (OCT) compound (Sakura Finetek, Zoeterwoude, the Netherlands), frozen instantly in liquid nitrogen and stored at -80°C until use.

Specimens embedded in Tissue Tek and frozen immediately at -80°C in liquid nitrogen were cut into 5-µm-thick sections, air-dried, fixed in acetone for 5 min and kept at -80°C. Endogenous peroxidase activity was blocked by incubation for 5 min in peroxidase block, diluted in 0.03% hydrogen peroxide in 95% ethanol. Following three rinses with distilled water, 0.05% Tris-buffered saline (TBS) for 5 min and 1% bovine serum albumin (BSA) in TBS for 10 min, the sections were incubated for 60 min at room temperature with the primary antibodies (mouse anti-human) diluted in 1% BSA/TBS in the following dilutions: anti-CD4 (clone 4B12; 1:20) and anti-CD8 (clone 1A5; 1:20) obtained from Novocastra and anti-forkhead box P3 (FoxP3) antibody (clone 236 A/E7; 1:50), obtained from eBioscience (San Diego, CA, USA). After rinsing with TBS, a secondary antibody (EnVision+ kit K4004; Dako, Carpinteria, CA, USA) labelled with horseradish peroxidase was applied for 30 min at room temperature. Enzymatic activity was revealed by a 5–10-min incubation with 3, 3'-diaminobenzidine (DAB) + substrate-chromogen (EnVision+ kit K4007; Dako), which results in a brown-coloured precipitate at the antigen site. Counterstaining was performed with aqueous Mayer's haematoxylin (Merck, Darmstadt, Germany).

Negative controls were performed with omission of the primary antibody.

The sections and antibodies were examined using an LSM 510 microscope (Carl Zeiss MicroImaging, Oberkochen, Germany).

Preparation of total RNA

Biopsies taken from 17 individuals, seven patients with psoriasis, two of whom had a positive elicitation reaction and 10 healthy controls, five of whom had a positive elicitation reaction, were prepared for the microarray study. Before taking these skin biopsies the skin was frozen using a liquid nitrogen spray to inhibit RNA degradation. The skin biopsies were placed immediately in liquid nitrogen and transferred to a -80°C freezer. For RNA extraction, the frozen skin biopsies were ground in liquid nitrogen, transferred to lysis/binding buffer (Applied Biosystems, Rotterdam, the Netherlands) and homogenized with a rotor stator (Polytron PT₃₀₀₀; Kinematica AG, Buch & Holm A/S, Herlev, Denmark). Total RNA was then extracted using the *mirVana*TM isolation kit (Applied Biosystems) following the manufacturer's specifications. RNA concentration was determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the RNA quality was assessed using an Agilent RNA 6000 nano kit on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The RNA was stored at -80°C .

DNA microarrays

The microarrays used for this study were Human Gene 1-0 ST arrays (Affymetrix Inc., Santa Clara, CA, USA) containing probe sets of approximately 26 000 genes.

Generation of cDNA, biotin-labelled cRNA and GeneChip hybridization was performed by the RH Microarray Centre at Rigshospitalet (Copenhagen, Denmark).

Samples were labelled according to the manufacturer's guidelines using GeneChip Whole Transcript Sense Target Labeling Assay (Affymetrix). In short, 100 ng of total RNA was transcribed into cDNA using T7-(N)₆ primers. Anti-sense RNA is generated by *in vitro* transcription of the cDNA, which is used as a template to generate 2'-deoxyuridine, 5'-triphosphate (dUTP)-containing sense cDNA fragments. Following the removal of RNA fragments, the sense cDNA fragments were terminal-labelled with biotin. The labelled samples were hybridized to the Human Gene 1-0 gene ST array (Affymetrix). The arrays were washed and stained with phycoerythrin-conjugated streptavidin (SAPE) using the Affymetrix Fluidics Station® 450, and the arrays were scanned in the Affymetrix GeneArray® 3000 scanner to generate fluorescent images, as described in the Affymetrix GeneChip® protocol.

Data analysis

Clinical data

Group sensitization rates were compared using a χ^2 test and logistic regression analyses. The challenge outcome, whether positive or negative, was used as the dependent variable and skin status, sex and age as the independent variables. Results were expressed as odds ratios (OR) with 95% confidence intervals (CI).

The strength of reactions in the three groups was analysed with both the visual scores and ultrasound data. The Mann–Whitney two-sample rank sum test was used to compare the sum clinical scores in the groups. Using the ultrasound data, linear regression analyses of the elicitation responses were calculated for each individual and the means of the slopes and the intercepts, used as parameters for strength of the elicitation reaction, were compared for each group using a Mann–Whitney *U*-test, as recommended for serial measurements. *P*-value < 0.05 was considered to be statistically significant.

All data analysis was performed using SPSS version 12 (SPSS, Inc., Chicago, IL, USA).

Immunohistochemistry data

The degree of infiltration of positively stained cells was scored semi-quantitatively using a five-point scale: 0 = none, 1 = few positive, 2 = some positive, 3 = many positive and 4 = highly positive.

To detect significant differences between the group mean values, the Mann–Whitney *U*-test was applied. Significance was determined with a *P*-value < 0.05. All data analysis was performed using SPSS version 12 (SPSS, Inc.).

Microarray data

Analysis of the microarray data followed the overall strategy described previously [12]. Briefly, a single log₂ scale expression measure for each probe set was attained from the low-level data files (CEL files), using the robust multi-array analysis procedure with quantile normalization [13] implemented in the Affymetrix library for the R statistical environment.

Principle component analysis (PCA) was carried out with the *prcomp* R function. The 2.5% of the probe sets with the most extreme positive or negative loading values for the first two principal components were extracted and used for analysis of over-represented GO terms using Fisher's exact test for proportions with Bonferroni's correction for multiple testing. Mapping of probe IDs to GO terms was performed with the *annaffy* package from Bioconductor [14].

To analyse further the possible differences in gene expression between psoriasis patients and healthy controls, probe sets from psoriasis patients with negative elicitation reac-

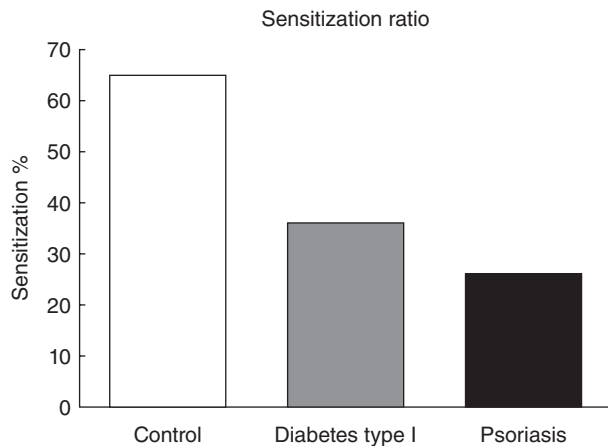


Fig. 1. Sensitization ratios of healthy controls ($n = 23$), diabetes type I patients ($n = 22$) and psoriasis patients ($n = 23$).

tions as well as healthy individuals, also with a negative elicitation reaction, were selected for further analysis using the *t*-test and subsequent correction for multiple testing with Bonferroni's adjustment.

Results

Sensitization outcome

Sensitization ratios were lower in both the psoriatic and diabetic groups compared to the healthy subjects group. The sensitization ratio was 26% (3:23) for the psoriatic group, 36% (8:22) for the diabetic group and 65% (15:23) for the healthy control group (Fig. 1). The logistic regression analysis for a psoriasis patient gave an OR of being sensitized to 0.18 (95% CI: 0.039–0.85), $P = 0.031$, when adjusted for sex and age. The crude OR of being sensitized for a diabetes type I patient was 0.74 (95% CI: 0.548–1.008), $P = 0.056$.

The percentage increase in dermal thickness, as measured by ultrasound, correlated well with the dose-dependent clinical scores of the visual assessment, and a linear dose-dependent increase in response to DPCP was seen in all positively sensitized individuals. The overall strength of the elicitation responses of positively sensitized individuals is summarized in Table 1. For sensitized individuals there were no statistically significant differences in strength of elicitation between the groups.

The challenge doses used did not show any irritant response in unsensitized individuals.

Immunohistochemistry

In all five biopsies from subjects with a positive elicitation reaction, including healthy controls and psoriasis patients, a typical histological pattern of allergic contact dermatitis was present. Apart from one single outlier, all five biopsies had a grade 4 infiltration of CD4⁺, CD8⁺ and FoxP3⁺ cells, as demonstrated in Fig. 2. CD4⁺ cells and FoxP3⁺ were distributed mainly in the dermis, with only scattered cells in the epidermis. CD8⁺ cells were also found mainly in the dermis, but with a higher degree of infiltration in the epidermis. The outlier was a healthy subject with a severe clinical reaction; her biopsies were with grade 4 infiltrations of CD8⁺ cells, but with very few CD4⁺ or FoxP3⁺ cells. The six biopsies from subjects with negative elicitation reactions all showed a histological picture of healthy skin; hence, there were no signs of subclinical reactions. All had a grade 1–2 degree of CD4⁺ cells, but no CD8⁺ cells and only a limited number of FoxP3⁺ cells.

No distinction between biopsies from healthy controls and psoriasis patients could be made from the infiltration of T cells in patients with either a positive or negative elicitation reaction.

Gene expression profiles

The whole data set and subsets thereof were subjected to PCA. Figure 3 depicts a score plot of the first two principal components of the PCA with DPCP-treated skin biopsies only. The first two dimensions retained 22 and 11% of the variation in the data set, respectively. A clear separation of the biopsies along the first dimension is observed. Biopsies from patients with negative clinical elicitation reaction are projected towards positive values in the first dimension, and biopsies from patients with clinical positive elicitation reaction are projected towards negative values. Thus, the first axis distinguishes the skin from patients with positive clinical elicitation reactions from patients with negative elicitation reactions. The group of psoriasis patients could not be distinguished in the PCA score plot from healthy individuals, regardless of clinical elicitation reactivity.

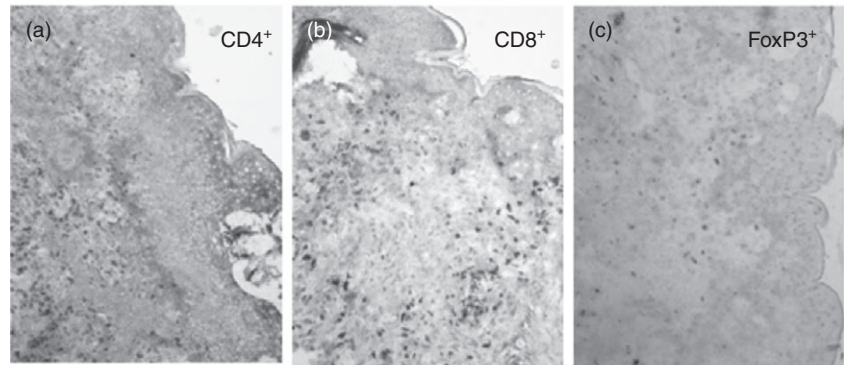
To identify the probe sets that define the positive and negative directions of the axes and identify significantly over-represented annotation terms, an annotation analysis was applied. Annotation terms for biological processes are defined by the Gene Ontology Consortium. The annotation analysis revealed that terms for biological processes related

Table 1. Strength of positive elicitation responses measured with visual clinical score and ultrasound.

Group	Positive	Sum clinical score [†]	Slope of UL regression line [†]	Intercept of UL regression line [†]
Healthy	15	9.7 ± 1.1	23.1 ± 3.69	59.0 ± 14.2
Psoriasis	6	8.3 ± 0.6	26.9 ± 5.25	35.8 ± 4.78
DM	8	9.7 ± 1.5	15.9 ± 2.88	51.6 ± 19.0

[†]Mean ± standard error of the mean. DM: diabetes mellitus; UL: ultrasound.

A positive elicitation reaction



A negative elicitation reaction

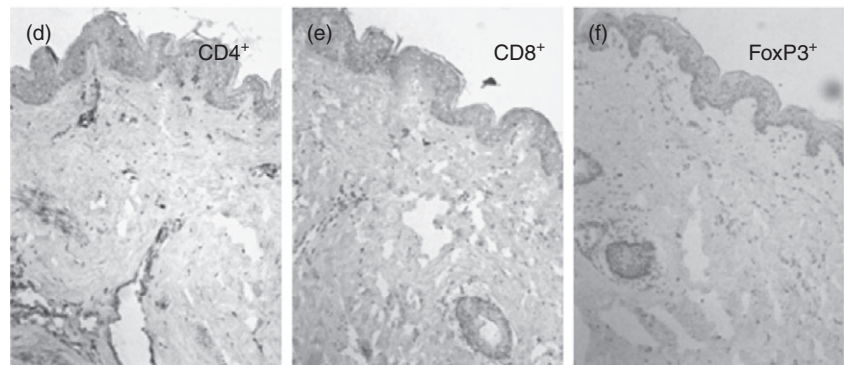


Fig. 2. Immunohistochemistry showing a positive elicitation reaction in the same healthy control subject. (a) CD4⁺ cells; (b) CD8⁺ cells; (c) forkhead box P3 (FoxP3⁺) cells and immunohistochemistry of a negative elicitation reaction in another subject; (d) CD4⁺ cells; (e) CD8⁺ cells; (f) FoxP3⁺ cells.

to immune response were over-represented in the annotation genes defining the negative direction of the first PC axis. The negative direction of PC1 represents the activation of genes as a result of the cellular response to the allergen, DPCP. In the annotation analysis 129 different GO terms

were found to be over-represented in genes up-regulated as a response to DPCP stimulation (clinical positive reactions). These GO terms were all related in some way to the inflammatory response and the genes annotated with the three most relevant terms are listed in Table 2. In contrast, the positive direction of PC1 represents the clinical negative elicitation reactions as well as the vehicle-stimulated skin, and consequently very few GO terms were found to be over-represented in genes associated with this direction of PC1. In fact, only one term (GO:0048856), 'Anatomical structure development', was found to be significantly over-represented. This term is very broad, and includes many thousands of gene products expressed in normal skin. To investigate further whether or not elicitation reactions were specifically down-regulated in psoriasis patients, probe sets from psoriasis patients with a negative elicitation reaction as well as healthy individuals also with a negative elicitation reaction were selected for further analysis using the *t*-test and subsequent correction for multiple testing with Bonferroni adjustment. When comparing the two groups, no significant difference was found in gene expression.

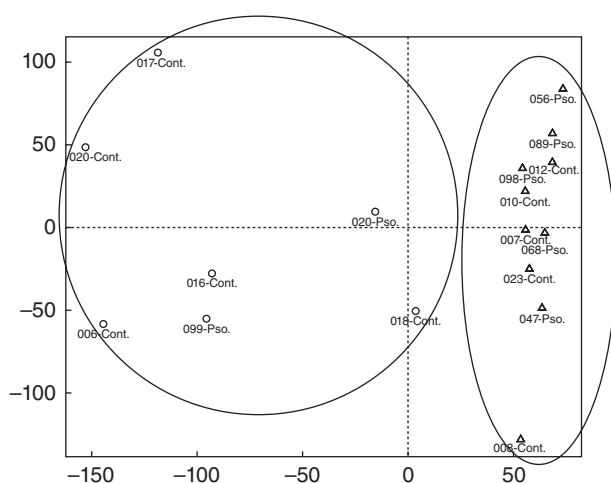


Fig. 3. Principle component analysis (PCA) plot, scores for the first and second axes. The projections of samples from subjects with positive (○) and negative (Δ) elicitation reactions, respectively, are indicated by circles.

Discussion

In a controlled experimental sensitization study using the strong allergen DPCP, with a sensitization potential stronger

Table 2. Genes with inflammation-related functions and increased expression during positive elicitation response (Fdr < 0.01).

GO term	Hugene10st identifier	Symbol	Description	Fold increase
Immune response GO:0006955	7908553	PTPRC	Protein tyrosine phosphatase, receptor C	4.78
	7945371	IFITM3	Interferon-induced transmembrane protein 3	2.03
	7952601	ETS1	V-ets erythroblastosis virus E26 oncogene	1.84
	7961532	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	1.98
	7971461	LCP1	Lymphocyte cytosolic protein 1 (L-plastin)	3.40
	7972557	GPR183	G protein-coupled receptor 183	5.26
	7982597	THBS1	Thrombospondin 1	4.70
	7994769	CORO1A	Coronin, actin binding protein 1A	3.05
	7994826	ITGAL	Integrin alpha L	3.18
	8003903	ARRB2	Arrestin beta 2	1.99
	8006433	CCL2	Chemokine (C-C motif) ligand 2	7.73
	8025601	ICAM1	Intercellular adhesion molecule 1	3.81
	8045539	KYNU	Kynureninase (L-kynurenine hydrolase)	3.94
	8047677	CD28	CD28 molecule	3.93
	8054722	IL1B	Interleukin 1 beta	1.98
	8078442	CCR4	Chemokine (C-C motif) receptor 4	4.11
	8079392	CCR2	Chemokine (C-C motif) receptor 2	4.08
	8086600	CCR1	Chemokine (C-C motif) receptor 1	5.15
	8093294	CCR2	Chemokine (C-C motif) receptor 2	4.25
	8104901	IL7R	Interleukin 7 receptor	11.01
	8115734	LCP2	Lymphocyte cytosolic protein 2	3.08
	8116559	IRF4	Interferon regulatory factor 4	2.07
	8178193	HLA-DRA	Major histocompatibility complex, class II	2.29
	8179481	HLA-DRA	Major histocompatibility complex, class II	2.07
Inflammatory response GO:0006954	7905571	S100A9	S100 calcium binding protein A9	13.41
	7920244	S100A8	S100 calcium binding protein A8	16.94
	7982597	THBS1	Thrombospondin 1	4.70
	7994826	ITGAL	Integrin alpha	3.18
	8054722	IL1B	Interleukin 1 beta	1.98
	8070826	ITGB2	Integrin beta 2	2.98
	8079392	CCR2	Chemokine (C-C motif) receptor 2	4.08
	8086600	CCR1	Chemokine (C-C motif) receptor 1	5.15
	8093294	CCR2	Chemokine (C-C motif) receptor 2	4.25
	8096635	NFKB1	Nuclear factor of kappa light polypeptide	1.68
	8105040	OSMR	Oncostatin M receptor	3.16
	8179112	ABCF1	ATP-binding cassette, subfamily F	1.33
T cell activation GO:0042110	7903358	VCAM1	Vascular cell adhesion molecule 1	4.85
	7908553	PTPRC	Protein tyrosine phosphatase, receptor C	4.78
	7944185	CD3G	CD3g molecule, gamma (CD3-TCR complex)	4.93
	7952056	CD3D	CD3d molecule, delta (CD3-TCR complex)	3.67
	7971461	LCP1	Lymphocyte cytosolic protein 1 (L-plastin)	3.40
	7994826	ITGAL	Integrin alpha L	3.18
	8025601	ICAM1	Intercellular adhesion molecule 1	3.81
	8047677	CD28	CD28 molecule	3.93
	8054722	IL1B	Interleukin 1 beta	1.98
	8116559	IRF4	Interferon regulatory factor 4	2.07

than most allergens encountered in the environment, we believe we are the first to show lower sensitization ratios in two groups of psoriasis and diabetes type I patients, respectively, compared with healthy controls. The results support recent epidemiological studies showing an inverse relation between contact allergy and several autoimmune diseases: psoriasis, diabetes type I, inflammatory bowel disease and rheumatoid arthritis [1–4].

In 1965 Epstein and Maibach sensitized 13 psoriasis patients and 32 healthy controls with the strong allergen DNCB and found a slightly reduced sensitization ratio in the psoriatic group, but interpretation was hampered by the small study sample [15]. Two other experimental studies sensitizing psoriatic patients with DNCB have been conducted. Both studies used a high allergen dose for sensitization, sensitizing almost all participants, and hence they

focused on the degree of challenge responses only. Moss *et al.* found reduced challenge reactions compared to healthy controls [5], and Obalek and co-workers reported a higher threshold in psoriasis patients compared to healthy controls [6]. These results strongly suggest changes in the elicitation phase of sensitization among psoriatic patients. We only found a trend towards reduced reactivity in challenge responses. This might be due to the use of a different allergen or, more probably, that the effect is dependent upon the sensitization dose, which in our study was deliberately chosen to be relatively low, sensitizing only 65% of the healthy group in order to study the differences in sensitization potentials.

A low sensitization ratio of patients with diabetes type I compared with healthy controls was found in our study, although on the border of statistical significance.

One study has demonstrated a reduced sensitization ratio in patients with rheumatoid arthritis using DNCB [7], indicating that the impaired reactivity to haptens could be common for autoimmune diseases. The autoimmune diseases psoriasis, diabetes type I, rheumatoid arthritis and inflammatory bowel disease have been linked through common clinical traits, genetic polymorphisms and immunological pathways [16–18]. Theoretically, it seems likely that the autoimmune diseases share an immunological milieu that can interfere with the expression of a contact allergic response.

In contact allergy an individual becomes sensitized to a hapten, a low molecular weight chemical, through a complex process involving integrated signals from the innate and adaptive immune system, in which during the induction phase T cells are primed in lymphoid organs, and upon re-exposure to the hapten during the elicitation phase are recruited to the skin and mediate the clinical outcome of allergic contact dermatitis. In murine studies, regulatory T cells have been shown to play a regulatory role in reducing the magnitude of the elicitation responses and in preventing priming to haptens [19–21]. In humans, specific CD4⁺CD25⁺ regulatory T cells capable of inhibiting CD4⁺CD25[−] nickel-specific effector T cells *in vitro* have been demonstrated in allergen-challenged skin and blood of non-allergic individuals [8,9], indicating an active down-regulation. These findings led us to investigate the elicitation sites of the participants in our sensitization study for down-regulatory mechanisms. Study samples were small and the study was regarded only as exploratory. Using immunohistochemical staining, we found no difference in cell infiltration between healthy skin and skin from challenge sites of non-responders after 48 h, indicating that the lack of response was not due to active down-regulation. In agreement with this, we did not find any significant up- or down-regulation of gene expression in the challenge sites of non-responders after 48 h. Furthermore, mRNA expression in elicitation responses of patients with psoriasis and healthy controls could not be distinguished, either in the positive or negative reactions,

indicating that the clinical difference between the groups is not due to a difference in down-regulation at the elicitation site. Regulatory T cells have been found to be dysfunctional in suppressing auto-antigen specific effector T cells in various autoimmune diseases [22–25], but their regulation of environmental antigens were not investigated in these studies. It is theoretically possible that some sort of down-regulatory event took place before 48 h at which the biopsy was taken; however, a cellular response would be expected to be present at this time-point.

The significance of a T helper type 17 (Th17) profile in autoimmune diseases is well established through multiple areas of research, as reviewed by Steinmann [26], and patients with autoimmune diseases have been demonstrated to have higher than normal levels of circulating Th17 cells and cytokines such as interleukin (IL)-17, IL-6, IL-21, IL-22 and IL-23 [27]. We hypothesize that the highly Th17-directed cytokine milieu in patients with autoimmune diseases interferes with the mounting of a contact allergic response. This could be due to interference in the differentiation of naive T cells to become effector or memory T cells necessary for the contact allergic reaction or to regulation of antigen-presenting cells. Interestingly, in a murine study, Brandt *et al.* demonstrated that short-term incubation of *in vitro*-generated dendritic cells (DC) with IL-21 significantly reduced their potential to induce an antigen-specific CD8⁺ T cell proliferation [28,29]. Antigen-presenting cells, particularly Langerhans cells, play a pivotal role in the sensitization phase of contact allergy, as they are responsible for the processing, transport and presentation of allergens to naive T lymphocytes in the skin, draining lymph nodes. Cumberbatch *et al.* found that the function of epidermal Langerhans cells, specifically Langerhans cells mobilization and migration, is profoundly impaired in the uninvolved skin of psoriasis patients compared with the skin of healthy volunteers [30]. The authors hypothesized that this could be due to disease progression characterized by systemic changes that affect Langerhans cell function. The systemic changes could be due to a Th17-skewed milieu found not only in psoriasis patients, but also in patients with other autoimmune diseases.

In conclusion, we found reduced sensitization ratios in patients with the autoimmune diseases psoriasis and type I diabetes and suggest that the immunological background is not to be found in the elicitation, but more probably in the sensitization phase of contact allergy.

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Disclosure

None.

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