Association between Contact allergy and Psoriasis

PhD thesis
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This PhD thesis is based on the following manuscripts:

I. **Bangsgaard N**, Engkilde K, Thyssen JP, Linneberg A, Nielsen NH, Menné T, Skov L, Johansen JD. Inverse relationship between contact allergy and psoriasis: results from a patient- and a population-based study. *Br J Dermatol*. 2009 Nov; 161(5):1119-23.

II. **Bangsgaard N**, Engkilde K, Menné T, Løvendorf M, Jacobsen GK, Olsen J, Skov L. Impaired hapten sensitization in patients with autoimmune disease; Results from a sensitization study. In prep.

III. **Bangsgaard N**, Carlsen BC, Johansen JD, Menné T, Skov L. Susceptibility and reactivity in polysensitized individuals following controlled induction. Contact Dermatitis. 2010 Jul; 63(1):10-4.

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PREFACE

The work of this phD thesis was conducted between November 2007 and September 2010 at the department of Dermato-Allergology, University Hospital of Copenhagen Gentofte under the excellent guidance of Professor Lone Skov, Professor Jeanne Duus Johansen and Professor Torkil Menné.
The thesis is based on the results from three research studies: an epidemiological study, a clinical study and an experimental study, presented in three manuscripts.
The epidemiological studies were done in successful cooperation with colleagues at the National Allergy Research Centre; Kåre Engkilde and Jacob Thyssen.
The immunohistochemical studies were carried out at the Bartholin institute, where laboratory assistance and supervision were kindly provided from Margit Bæksted and Grete Kragh Jacobsen.
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ABBREVIATIONS

ACD: Allergic Contact Dermatitis

CHS: Contact Hypersensitivity

DNCB: Dinitrochlorobenzene

DPCP: Diphenylcyclopropenone

RA: Rheumatoid Arthritis

NDMA: N-Nitrosodimethylamine

DNHR: Danish National Hospital Registry

PCA: Principal Component Analysis

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1. BACKGROUND

1.1. Epidemiology

1.1.1. Contact allergy

Allergic contact dermatitis (ACD) is an inflammatory skin disease. It is the clinical manifestation of contact allergy, conditioned by skin contact with sensitizing molecules in the environment such as metals, perfumes and preservatives. Contact allergy is affecting 15-20% of the general population (1). Up to half of these cases have had clinical symptoms (2, 3), which makes ACD one of the most prevalent inflammatory skin diseases in western countries and a major burden to the individual as well as society as a whole.
The typical clinical appearance of ACD is a pruritic eruption with erythema, blistering and weeping, which in the chronic phase develop into lichenified or scaly plaques. The histopathological appearance of ACD is dominated by spongiosis, at times with intraepidermal vesiculation together with a mixed dermal inflammatory infiltrate (4).
The diagnosis of contact allergy is based on a positive epicutanous patch-test. In Europe the European Baseline Series are mainly used, currently including 28 allergens. The reproducibility of the patch-test is generally high but allergen dependent (5).

1.1.2. Psoriasis

Psoriasis is a prevalent, chronic skin disease affecting approximately 2-3% of the general western population (6, 7). Psoriasis Vulgaris is the most common variant accounting for 90% of all cases. Psoriasis can occur at any age but usually manifest itself between the age of 15 and 30 years by the conversion of symptomless skin into well-demarcated, thick, erythematous plaques with adherent silvery scales (8). The most specific histopathological changes are dramatic hyperplasia of the epidermis (acanthosis) with loss of the granular layer, regular elongation of the rete ridges, thickening of the cornified layer (hyperkeratosis) and incomplete keratinocyte differentiation (parakeratosis), infiltration of many different leukocytes and increased vascularity in the dermis (9).
The disease has a strong genetic component with a 40–70% concordance in identical twins (10, 11). More than 60% of psoriasis patients carry one or two class I HLA-Cw*0602 alleles compared with a population frequency of 10–15%, and this HLA allele was recently strongly implicated as an important susceptibility allele in psoriasis (12,13).

Although a self-antigen responsible for the development of psoriasis has not been identified, psoriasis is generally considered to be an autoimmune disease. In epidemiological studies psoriasis has been shown to be associated with other autoimmune diseases such as rheumatoid arthritis, Crohn´s disease and diabetes Type I, more frequently than expected and to share genetic susceptibility loci (14, 15). The disorders represent separate entities but appear to follow overlapping pathogenic pathways (16).

1.2. Effector & regulatory mechanisms

1.2.1. Contact allergy

Contact allergy, also termed Contact Hypersensitivity (CHS), is classically designated as a type IV hypersensitivity reaction primarily mediated by T cells. CHS consists of two distinct phases, a sensitisation phase and an elicitation phase. The sensitisation phase includes the events following first contact with an allergen by which an individual becomes sensitized due to the development of immunological memory. Following primary allergen application to the skin, antigen presenting cells take up the hapten, process it and migrate towards the regional lymph nodes, where the antigen is presented to naïve T cells and hapten specific CD4+ Th1-biased and CD8+ cytotoxic effector T cells are generated. The induction phase is often symptomless and typically last 10–15 days. The elicitation phase begins when a sensitized individual is re-exposed to the allergen and the memory T cells are recruited to the skin leading to clinical manifestation of ACD; this phase typically takes 72 hours but may vary (17, 18).

The main effector T helper cell subset in contact allergy is the Th1 cell. Although Th17 cells and IL-17 have been demonstrated in contact allergy, the importance of this remains unknown (See figure 1. for a brief schematic overview of T helper subsets).

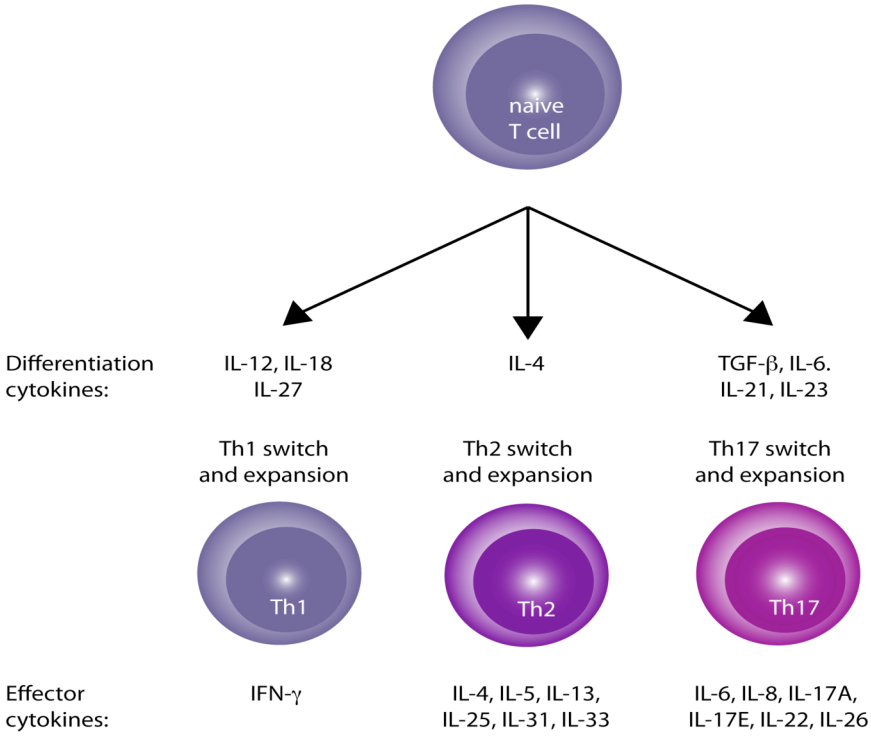


FIGURE 1: Brief overview of Th1, Th2 and Th17 cell differentiation including differentiation and effector cytokines.

Most individuals are in contact with potential sensitizers in everyday life without developing contact allergy and the degree of contact allergy in allergic individuals can fluctuate. Many studies have suggested that the reaction of contact allergy is highly regulated, in part due to regulatory T cells. Regulatory T cells are a heterogeneous family of naturally occurring and peripherally induced specialised T cells, which suppress immune responses by releasing anti-inflammatory cytokines or by inactivating effector T cells through cell-to-cell contact. (18). See diagram in figure 2 for a brief schematic overview.

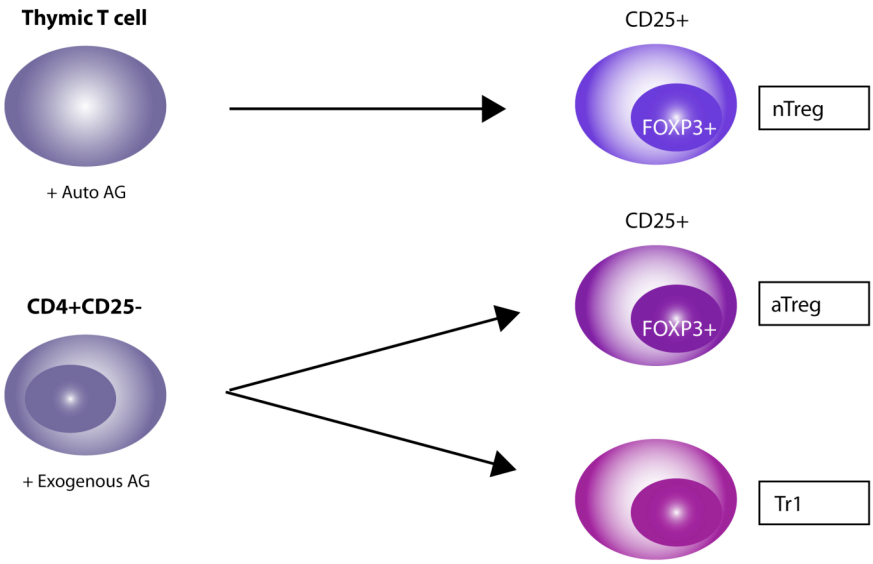


FIGURE 2: Brief overview of well described regulatory T cells. nTreg is naturally occurring, generated in the Thymus. aTreg is adaptive and generated in the periphery.

Both Tr1 and FOXP3+ regulatory T cells have been associated with contact allergy. The role of Tr1 cells in down-regulation of the elicitation phase of contact allergy has been demonstrated in human studies by Sebastiani et al (19). Tr1 cells isolated from blood of nickel allergic individuals were shown to down-regulate the immune response to nickel through the release of high amounts of IL-10 acting on APCs by blocking their IL-12 release and antigen presenting functions. It was demonstrated that Tr1 recruitment at the site of nickel application was mostly promoted by the local release of the chemokine CCL1. CCL1 was released by keratinocytes and infiltrated leukocytes late during ACD reaction, suggesting that Tr1 cells may be involved in the termination of the immune response to avoid excessive tissue damage (20).

In a murine study CD4+CD25+FOXP3+ regulatory T cells were reported to suppress ACD reaction by blocking influx of effector T cells into inflamed tissue (21), and in humans allergen specific CD4+CD25+ regulatory T cells have been found in allergen challenged skin and blood of non-allergic individuals (22, 23), indicating an active down-regulation. Furthermore, it was found that CD4+CD25+ regulatory T cells from nickel allergic

individuals had limited capacity to down-regulate nickel specific effector T cell function, suggesting that the ACD could be due to a defective regulatory T cell function (22).
The relative contributions of different regulatory T cell subsets in the control of ACD reactions still need to be established and many aspects of the functional relevance of effector and regulatory T cells in contact allergy are still not thoroughly understood.

1.2.2. Psoriasis

The exact pathophysiological mechanism of psoriasis has not been determined; however, disease initiation is suggested to be mediated through dendritic cells presenting self-antigens to T lymphocytes that mediate the pathological process in genetically predisposed individuals (24). In support of this hypothesis, activated T cells from psoriasis patients, but not healthy controls, have been found to have the capacity to induce psoriasis-like changes when injected into human skin samples transplanted onto SCID mice (25). Also, T cell clones from psoriatic skin lesions have been shown to be able to enhance the proliferation of keratinocytes, (26-28) demonstrating the pivotal role of the T cell in psoriasis.

The autoimmune diseases have originally been ascribed to Th1 cell activity with the production of IFN-γ and TNF-α as the main pro-inflammatory mediators; however, this has been questioned in recent years with the finding of a new Th cell subset in autoimmune diseases, the IL-17 secreting Th17 cell.

Accumulating evidence from human and mouse studies suggest that the Th17 subset is an important mediator during pathogenesis of autoimmune diseases, including psoriasis (29). Increased numbers of Th17 cells have been demonstrated in psoriatic lesion (30), IL-17 mRNA has been shown to increase with disease activity (31) and a significant elevation in circulating Th17 cells in patients with psoriasis has been found (32).
The development and maintenance of Th17 cells have been linked to IL-23, a key initiating cytokine, in the development of autoimmunity (33, 34). In psoriasis elevated levels of IL-23 have been found in serum and cutaneous lesions of patients with psoriasis. Therapies targeting IL-23 have shown promising treatment results (35) and genes involved in IL-23 signalling have been associated with psoriasis (36-38), all supporting an importance of a

Th17/IL-23 pathway in psoriasis. The current understanding is that psoriasis is a mixed Th1 and Th17 inflammatory environment.

The role of regulatory T cells in autoimmune diseases, as in other inflammatory diseases, has received a great deal of attention in recent years. Under conditions of healthy immune homeostasis, the activation of effector T cells against self-antigen is controlled by naturally occurring CD4+Foxp3+CD25+ regulatory T cells (39). The importance of this is observed in FOXP3 knockout mice, which at an early age develop fatal multi-organ autoimmunity due to the lack of regulatory T cells (40), and in patients with FOXP3 mutations that develop IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome associated with severe autoimmunity (41). Dysfunction of the regulatory T cell population is an obvious potential explanation for the unrestrained pathogenic/effector T cell proliferation seen in autoimmune diseases and indeed regulatory T cell dysfunction has been demonstrated in varies autoimmune diseases (42-45). In psoriasis regulatory T cells isolated from psoriatic patients have been found less capable than those from healthy controls of suppressing proliferation of responder T cells isolated from healthy and psoriatic subjects (42). The dysfunction was demonstrated in relation to allo-antigen induced T cell stimulation. The function of regulatory T cells activated due to environmental antigens in patients with psoriasis was not investigated.
The exact role and phenotype of regulatory T cells in psoriasis remain unclarified.

1.3. Association between contact allergy and psoriasis

The idea of an inverse association of contact allergy and psoriasis was studied in the 1960´s, but has since received limited attention. Using a large database of dermatological patients, Henseler et al. investigated disease concomitance in psoriasis (46). Contact Dermatitis was found to be three times less frequent in patients with psoriasis compared to a control group of patients with non-psoriatic skin diseases. However, in other studies in which patch testing of patients with psoriasis have been compared with different control groups the overall prevalence of contact allergy in patients with psoriasis has been reported to be at around 20–25% (47–49) and even as high as 68% (50).

Fedler et al. patch tested 552 adults, 88 of whom had psoriasis, with standard allergens and found that patients with psoriasis had significantly less nickel allergy than healthy controls, but found no difference in the overall prevalence of contact allergy (47). Barile et al. patch tested 305 patients with psoriasis and 96 patients with dermatological diseases, other than ACD and psoriasis, and found no difference in responsiveness to patch testing between the groups (48). In a recent study from Vojvodina, 56 patients with psoriasis and healthy controls were patch tested with the European Baseline Series (49) with no overall difference found.
To conclude, epidemiological studies have produced conflicting results and the association between contact allergy and psoriasis is unclear.

Three experimental sensitization studies with psoriatic patients have previously been conducted. In all three studies the strong allergen Dinitorchlorobenzene (DNCB) was used to sensitize patients with psoriasis and healthy controls. Three to four weeks later subjects were challenged (51-53). Sensitization studies can reveal sensitization potential, reactivity and threshold to the allergen in a specific group of patients depending on the study design. In two of the studies, one by Moss et al. and another by Obalek et al. (51, 52) a high induction dose of DNCB that sensitized 100% of all participants was used, hence the challenge potential of the groups was investigated, not the sensitization. The degree of reactivity to challenge in the study by Moss et al. was found to be reduced in patients with psoriasis (51). In the study by Obalek et al. higher thresholds were found in patients with psoriasis compared to healthy controls (52). In the third study a lower induction dose of DNCB was used (53) sensitizing only 66% and 42% of the healthy control and psoriatic group respectively. Unfortunately interpretation was hampered by the small study group; hence the sensitization potential of patients with psoriasis remains inconclusive.

Accumulating knowledge on molecular mechanisms, including regulation of the inflammatory diseases, has awakened the interest of an inverse relation between contact allergy and not only psoriasis but other autoimmune diseases. Recent epidemiological studies have showed an inverse association between contact allergy and the autoimmune diseases Diabetes type I, Crohns disease and rheumatoid arthritis (RA) (54-56). It is possible that a changed reaction towards haptens is a common feature for autoimmune

diseases. This hypothesis is supported by a study from 1959 (57) in which Jessar et al. sensitized 77 patients with RA with DNCB and the weaker allergen N-Nitrosodimethylamine (NDMA). The group found a reduced sensitization ratio among patients with RA, although only significantly for NDMA. When the sensitisation dose was lowered the difference in sensitization ratio between RA patients and healthy controls were increased. Sensitizations studies of patients with other autoimmune diseases have not been conducted and possible mechanisms behind the impaired hapten reactivity in patients with autoimmune diseases have not previously been investigated.

2. AIMS OF THE THESIS

Study part I – Epidemiological studies

To examine a possible association between contact allergy and psoriasis in two conceptually different epidemiological studies:

1. A patient register-based study
2. A population-based study

Study part II – Sensitization study

To investigate the potential of hapten sensitization and reactivity to challenge in patients with psoriasis and compare it with:

1. Patients with a different autoimmune disease; diabetes type I
2. Patients with mono-allergy or poly-allergy
3. Healthy controls

Study part III – Experimental studies

To explore the possibility of down-regulatory events in the elicitation phase by investigating skin biopsies taken from challenge sites with:

1. Immunohistochemistry
2. Messenger RNA expression profiles with microarray analysis

3. RESULTS & DISCUSSION

3.1 Study part I – Association between contact allergy and psoriasis

In the first study we investigated the association between contact allergy and psoriasis in two conceptually different epidemiological studies, a clinic-based register study and a population-based cross-sectional study. Previous epidemiological studies have produced conflicting results (46-50).

3.1.1 Association between contact allergy and psoriasis in a hospital population

Data for the clinic-based register study was obtained by linkage of patch-test results from a large database at the department of Dermato-allergology, Copenhagen University Hospital Gentofte, Denmark with a diagnosis of psoriasis from the Danish National Hospital Registry (DNHR), holding information on all discharges from Danish hospitals.
Between 1985 and 2006, 15,641 patients were patch tested. More females than males were tested in this period (63.7% vs. 36.3%). The age, defined as the age at the first positive patch test or the first patch test if all negative, spanned from 4 to 99 years with a median of 47. In 5,572 patients a positive outcome of patch-tests were found (35.6%). There were more positive outcomes among females than males (41.1% vs. 26.0%).
Of the 15,641 patients 806 had an ICD code for psoriasis (ICD8 69609, 69610, 69619 or ICD10 DL40). An outline of the linked register data is shown in table 1.
An inverse association was found between a psoriasis diagnosis and a positive patch-test. The crude odds ratio for a person with a psoriasis diagnosis of having a positive patch-test was 0.58 (CI 95% 0.49 - 0.68). The result did not change when adjustment for sex and age was applied (table 2).

	Negative patch-test (n=10,069)					Positive patch-test (n=5,572)				
Age (years)	0–15	16–30	31–50	51–70	>70	0–15	16–30	31–50	51–70	>70
Psoriasis	10	79	206	234	77	0	18	73	81	28
Not psoriasis	213	2096	3129	2719	1306	51	941	2039	1622	719

TABLE 1: Overview of contact allergy in the patch-test register

	P-value	Odds ratio	95% CI of odds ratio
Psoriasis	<0.0001	0.581	0.494 - 0.684
Psoriasis adjusted for sex and age	< 0.0001	0. 581	0.492 – 0.685

TABLE 2: Results from the logistic regressions on the clinic-based study with contact allergy as the response variable and psoriasis as the independent variable.

3.1.2. Methodological considerations, register study

The identification of patients with psoriasis was based on data from the DNHR. This register has been validated in several studies for other diagnosis and found to be between 66% and 99% accurate (58). Out-patients were not included in the DNHR until 1 January 1995. This could result in under-registration of patients with psoriasis. However, patients registered as out-patients from 1995 were all accounted for as in-patients. This supports the notion that being followed in a hospital department as out-patient will lead to admission at some point. It also reflects the fact that patients with psoriasis in the register population have moderate to severe psoriasis.

A difference in the pattern of referral to patch testing, for patients with psoriasis compared with that of patients with dermatitis, could result in a bias. According to the Berkson Bias being followed at a hospital increases the risk of being referred to testing. If such a difference exists it would be assumed to be more restricted for patients with psoriasis than for patients with dermatitis, which would lead to a negative rather than a positive association and so would make the found negative association even stronger.

UV-light or systemic immunosuppressant treatment for psoriasis could suppress a positive reaction at the patch testing. However, these treatments are as standard discontinued at least four weeks before testing and are therefore unlikely to have interfered with the result. Theoretically, patients with psoriasis could have a specific pattern of exposure to allergens; however, the relative distribution of allergies in patients with psoriasis in our material does not differ from the rest of the patients, which argues against a different exposure pattern for patients with psoriasis.

3.1.3. Association between contact allergy and psoriasis in a general population

Data for the population-based cross-sectional study was obtained from three consecutive cross-sectional studies conducted in Glostrup, Denmark in 1990, 1998 and 2006. Participants were patch tested and had completed a postal questionnaire asking the question: “has your doctor ever told you that you suffer from psoriasis?”
Between 1990 and 2006, 4,989 (98.5%) of 5,065 participants were patch tested. 616 (12.3%) of these had a positive patch test reaction to at least one allergen. 301 (6.1%) of 4,989 respondents reported that their doctor had at some point told them that they suffered from psoriasis. Logistic regression analysis revealed an inverse association between self-reported psoriasis and a positive patch-test reaction to at least one allergen with an odds ratio of 0.67 (CI 95% 0.44 - 1.00). After adjustment for sex and age group had been added the odds ratio was 0.64 (CI 95% 0.42 - 0.98) (table 3).

	P-value	Odds ratio	95% CI of odds ratio
Psoriasis	0.049	0.67	0.44 – 1.00
Psoriasis adjusted for sex and age	0.038	0.64	0.42 – 0.98

TABLE 3: Results from the logistic regressions on the population-based cross-sectional study with contact allergy as the response variable and psoriasis as the independent variable.

3.1.4. Methodological considerations, population study

The data are based on a large cross sectional study, which is a major strength. The possible limitations lie in the accuracy of the diagnoses. The diagnosis of psoriasis was obtained by use of a questionnaire. Self-reported diagnoses of different skin diseases tend to underestimate the prevalence (59); however, this does not seem to be the case in our study. Prevalence studies of psoriasis have reported 2–3 % in the general population (7, 8). With a high prevalence of psoriasis in the dataset of 6%, most people with the diagnosis of psoriasis are expected to be accounted for. It is possible that some participants incorrectly reported psoriasis instead of dermatitis; however, this would tend to underestimate the inverse association.

The diagnosis of contact allergy was based on patch testing. The prevalence of a positive patch-test reaction to at least one allergen was 12.3%, which is relatively low compared to previous reports from the general population (1). This is partly believed to be due to day 2 readings only. The readings have been the same for patients with and without psoriasis and are therefore not likely to have influenced the found inverse association.
Although not all invited subjects completed the questionnaire or took part in patch testing, the problem of non-respondense bias is not believed to be of any concern. If the non-respondense has lead to a bias, it would be due to participants with psoriasis having a different pattern of participation, related to their allergy-status, to participants without psoriasis, which is difficult to accept.

3.1.5 Result specific discussion

The association of psoriasis and contact allergy has previously been investigated using different approaches. Using a large database of dermatological patients, Henseler et al. investigated disease concomitance in psoriasis (46). ACD was found to be three times less frequent in patients with psoriasis compared to a control group of patients with non-psoriatic skin diseases. The patients were not patch tested and it could be argued that some of the patients with psoriasis may have had undiagnosed contact allergy. Conflicting results have been presented in studies in which patch testing of patients with psoriasis have been compared with control groups (47-50). The different results reveal difficulties in conducting such patient studies. These difficulties can be attributed to insufficient sample

size, inaccuracy of the psoriasis diagnosis, differences in patch testing technique and reading of results, alongside confounding factors such as UV and systemic treatment.

It is not possible from these studies to conclude if psoriasis is affecting the ability to develop contact allergy or whether having contact allergy inhibits the development of psoriasis. Unlike contact allergy, psoriasis has a strong genetic background but is likely to appear between the age of 15 and 30 years (60). This is the age that onset of many contact allergies are seen and so it is difficult to asses the causality in the found inverse relation. It is possible that the causation is bidirectional; that the two diseases mutually affect each other. Investigating one or the other requires different approaches. In the next study part we have, therefore, focused on how psoriasis affects the ability to develop contact allergy.

3.2 Study Part II - Sensitization potential and reactivity in patients with psoriasis

In the second study we investigated the potential of hapten sensitization and reactivity to challenge in patients with psoriasis compared with patients with diabetes type I, healthy controls, mono-allergic and poly-allergic individuals in a sensitization study. Diphenylcyclopropenone (DPCP), a strong allergen in a relatively low dose (25 µg), was used for sensitization on buttock skin. Challenge with four different doses of DPCP and an acetone control was carried out three weeks later on the upper inner arm. Challenge reactions were evaluated visually and with ultrasound.

A few studies have previously investigated sensitization of patients with psoriasis and reported reduced reactivity to challenge reactions (51-53). The focus of this study was mainly to investigate the ability of patients with psoriasis to become sensitized, which remain unclear.

3.2.1 Sensitization ratios

The percentage increase in dermal thickness, 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 sensitized individuals. A visual score of 2 or above, or a mean percentage increase in dermal thickness of 10% or more, was considered a positive reaction.

Sensitization ratios are shown in fig. 3. Sensitization ratios were lower in the psoriatic 26% (6/23) and diabetic group 36% (8/22) compared with the healthy 65% (15/23), mono-allergic 59% (13/22) and poly-allergic 57% (12/21) groups, which all had equal sensitization-ratios. The logistic regression analysis gave a crude odds ratio for a person with psoriasis of being sensitized to 0.18 (CI 95% 0. 039 - 0.85) P=0.031 compared to healthy controls. The crude odds ratio for a person with diabetes type I of being sensitized was 0.74 (95% CI 0.548 – 1.008) P= 0.056.

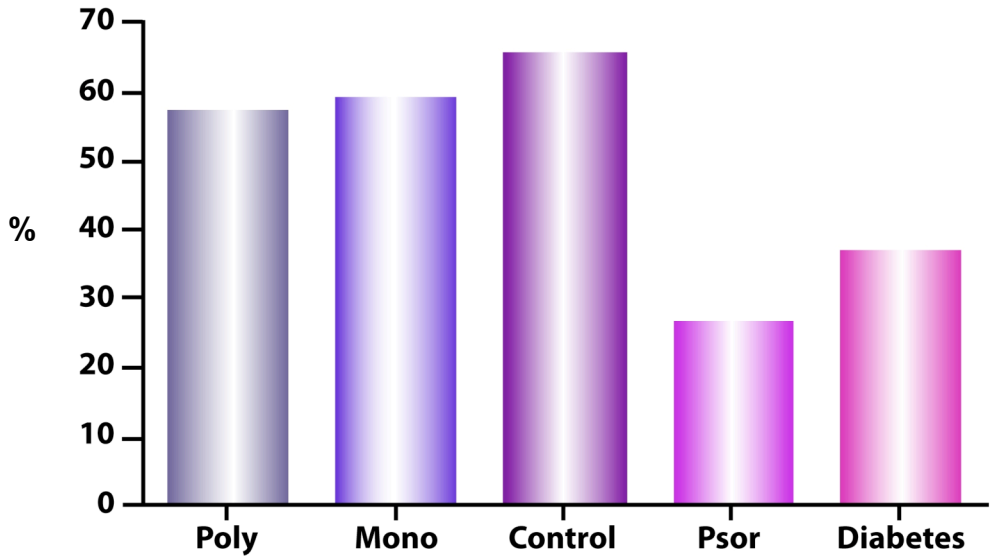


FIGURE 3: Sensitization ratios of poly-sensitized (n=21), mono-sensitized (n=22), healthy controls (n=23), patients with psoriasis (n=23) and patients with diabetes type I (n=22).

3.2.2 Reactivity to challenge

The overall strength of the elicitation responses were analysed with the visual clinical scores from 1 to 5, as well as with the increase in dermal thickness measured with ultrasound. Only subjects with a positive challenge reaction, as defined above, were included in the analysis; results are summarized in table 4. No statistically significant difference between the groups was found with any of the measurements, but a trend towards lower reactivity in the psoriatic group and a higher reactivity in the poly-allergic group was seen.

Threshold concentration was defined as the weakest concentration giving a positive response, with either visual clinical score or ultrasound. Threshold data for the groups is shown in Fig. 4. There was a significantly lower median elicitation threshold dose in the polysensitized group (0. 39 µg) compared to the mono-sensitized (1. 56 µg) and healthy control group (1.56 µg), with p=0.005 and p=0.037 respectively. Threshold for the other groups were equal.

Group	Pos.Sens	Sum Clinical Score +/-SEM	UL regression line +/- SEM	UL regression line +/- SEM
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
Mono	13	8.5 +/- 0.7	18.3 +/- 2.59	28.1 +/- 7.44
Poly	12	10.7 +/- 0.9	21.8 +/- 3.96	45.8 +/- 9.63

TABLE 4: Response to four increasing challenge doses of DPCP as assessed by visual clinical scores and UL. Only subjects with positive challenge reactions were included. Group mean +/- SEM indicated.

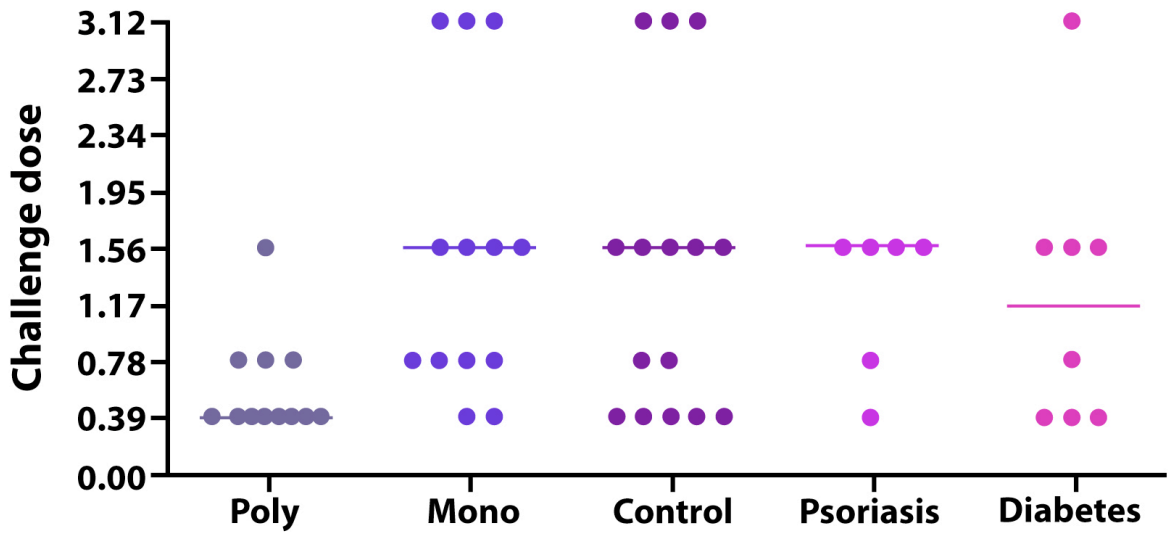


FIGURE 4: Elicitation thresholds of poly-sensitized (n=21), mono-sensitized (n=22), healthy controls (n=23), patients with psoriasis (n=23) and patients with diabetes type I (n=22). Group median is indicated.

3.2.3 Methodological considerations, sensitisation study

The sensitization protocol was adopted from Friedmann (61) in the version of Cooper and co (62). We used a different allergen, the strong allergen DPCP. Pivotal for the result is the standardization of sensitization and challenge doses, area of application and time of evaluation. We deliberately used a relatively low induction dose in order to investigate a possible difference in sensitization potential between the groups; thus being able to demonstrate a spectrum of lower and higher sensitization ratios than that of healthy controls.

None of the participants were under any kind of immunosuppressive or UV-light treatment, which is known to reduce sensitization (63). Experiments were completed winter and autumn when exposure to sun light is negligible. None of the participants in the study had a history of atopy, which could have affected the visual assessment, as atopic individuals have been shown to produce more vigorous irritant reactions (64). Prior to the experiment, patients with mono-allergy and poly-allergy selected for the study had all been patch tested at our department with positive results; however, we did not perform patch tests on controls, patients with psoriasis or patients with diabetes before inclusion. Some of the participants in these groups may have had an undiagnosed contact allergy, which would tend to underestimate any difference. 11 patients withdrew from the study after induction with DPCP on buttock skin; this poses a potential bias of results. However, the drop-outs were evenly distributed between the groups (3 controls, 1 mono-allergic, 3 poly-allergic, 2 with psoriasis, and 2 with diabetes) and only a few due to severe primary reactions.

The visual assessment of challenge reactions was not blinded and hence subjectivity may have skewed the result; however it is not likely to have influenced the results as assessment of challenge reactions was also done using UL and the percentage increase in dermal thickness, measured by ultrasound, correlated well with the dose-dependent clinical scores of the visual assessment.

3.2.4 Result specific discussion

The reduced sensitization ratio in patients with psoriasis and patients with diabetes type I is supporting the findings of the epidemiological studies in Part I and recent epidemiological studies showing an inverse relation between contact allergy and several autoimmune diseases; diabetes type I, inflammatory bowel disease and rheumatoid arthritis (54-56).

As mentioned in the introduction, three sensitization studies with psoriatic patients have been conducted previously. All used DNCB, a different but equally strong allergen, compared with DPCP as used in our study. Only one study investigated the ability of patients with psoriasis to become sensitized, by using a low sensitization dose, and found a slightly decreased sensitization ratio for patients with psoriasis compared to controls, 42% vs. 66% respectively (53). Unfortunately interpretation was hampered by the small study sample of patients with psoriasis n= 13. We used a sensitization dose that sensitized an equivalent proportion of healthy individuals with DPCP and found a much lower sensitization ratio in the psoriatic group. Differences in allergen application are not likely to account for this as DPCP and DNCB are both strong experimental allergens that subjects have not been exposed to before. The difference in results are more likely due to this study's much larger study group of patients with psoriasis n=23.
A low sensitization ratio in patients with RA has previously been demonstrated in a sensitization study (57), but sensitization studies on patients with diabetes have never been conducted.

The other two sensitization studies with psoriatic patients used sensitization doses that sensitized 100% of participants, hence a possible difference in sensitization potential would not be revealed (51, 52). Moss et al. found reduced challenge reactions in the psoriatic group compared to the healthy controls with number of patients and age of participants comparable to our study. Obalek et al. found a higher threshold in patients with psoriasis compared to healthy controls, but when high challenge doses were used no difference was found. These results strongly suggest changes in the elicitation phase among psoriatic patients.

We only found a trend towards reduced reactivity in challenge responses. The difference in results may be due to the use of a different allergen or, more likely, 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 reveal differences in sensitization potential. This is probably the same reason why we only found a trend towards higher reactivity to challenge in the poly-allergic group compared to a study by Moss et al. in which a higher reactivity was found using a dose sensitizing 100% with DNCB (65).

Positive sensitization is defined by a positive reaction to challenge. Bearing in mind that Obalek et al. found a higher threshold in patients with psoriasis than healthy controls, it is possible that using a stronger challenge dose would have revealed sensitization in some of the psoriatic patients, resulting in a higher sensitization ratio. However, the highest challenge dose of 3.125 resulted in severe reactions in many of the participants and is considered the highest acceptable concentration.

The sensitizations studies showed a reduced sensitization ratio and a trend towards reduced reactivity to challenge in patients with psoriasis compared to controls, but do not reveal the reason for this. In the next study we have focused on the mechanisms behind the impaired challenge responses found in patients with psoriasis.

3.3 Study Part III – Regulatory mechanisms in elicitation

In Study Part III we studied skin biopsies, taken from challenge sites of patients with psoriasis and healthy controls with immunohistochemistry and gene expression profiles, using microarray technology for possible down-regulatory mechanisms in patients with psoriasis. No study exploring the mechanisms behind a reduced reactivity to haptens in patients with psoriasis has previously been performed.

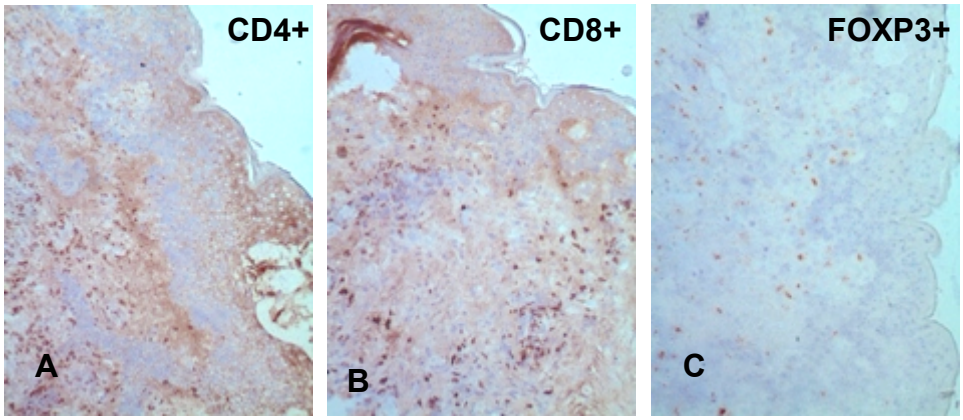
3.3.1 T cells in challenge sites

Biopsies were 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. The biopsies were prepared for immunohistochemistry and incubated with anti-CD4, anti-CD8 and anti-FOXP3 antibodies. The degree of infiltration of positively stained cells was scored semi quantitatively using a 5-point scale (grade 0–4).

In all five biopsies from subjects with a positive elicitation reaction, including healthy controls and patients with psoriasis, a typical histological pattern of ACD was present (an example is shown in figure 5. A-C). Apart from one single outlier, all five biopsies had a grade 4 infiltration of CD4+, CD8+ and FOXP3+ cells. CD4+ cells and FOXP3+ were mainly distributed in the dermis with only scattered cells in epidermis. CD8+ cells were also mainly found in dermis but with a higher degree of infiltration in the epidermis. The outlier was a healthy subject with a severe clinical reaction; her biopsies had 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 sign of subclinical reactions. All had a grade 1-2 degree of CD4+ cells, no CD8+ cells and only a limited number of FOXP3+ cells (an example is shown in figure 5. D-F).

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

A positive elicitation reaction



A negative elicitation reaction

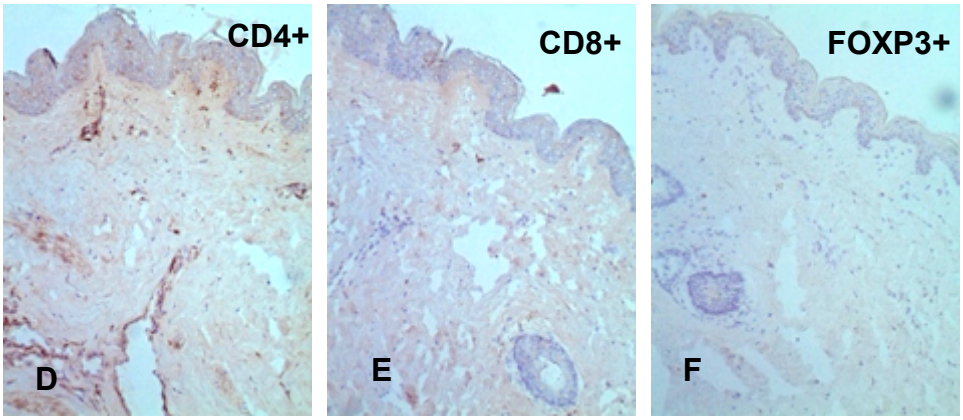


FIGURE 5: Immunohistochemistry showing a positive elicitation reaction in an allergic subject (A-C from the same subject) A) CD4+ positive cells B) CD8+ positive cells B) FOXP3+ positive cells and immunohistochemistry of a negative elicitation reaction in a non-allergic subject (D-F from the same subject) D) CD4+ positive cells E) CD8+ positive cells F) FOXP3+ positive cells.

3.3.2 Methodological considerations, immunohistochemistry

The visualisation of regulatory T cells has been hampered by the lack of a unique maker. At the initiation of this study FOXP3 was the best marker for regulatory T cells. FOXP3 belongs to the forkhead/winge-helix family of transcriptional regulators and appears to function as the master regulator in the development of regulatory T cells. FOXP3 is, however, also displayed transiently by recently activated effector T cells and is therefore not a unique marker of regulatory T cells (66).

The immunohistochemical studies were made on frozen tissue so not to exclude the possible use of new antibodies that cannot be used on paraffin embedded tissue. Preservation of tissue in paraffin usually gives a better histological picture with more details and some antibodies visualised with more specificity. However, the frozen sections in this study gave rise to standard histological pictures and clear antibody visualization. The gain of choosing paraffin embedded preservation instead is considered to be limited.

The immunohistochemical studies were conducted with a small study sample and should be regarded as exploratory only.

3.3.3 Gene expression profiles

In order to investigate possible differences in challenge responses between patients with psoriasis and healthy controls, biopsies from both groups, with and without positive challenge reactions, were selected for gene expression analysis. Seven patients with psoriasis, two of whom had a positive elicitation reaction, and ten healthy controls, five of whom had a positive elicitation reaction, were recruited. Messenger RNA expression profile analysis was carried out on RNA extracted from skin biopsies from challenge sites. A Principle Component Analysis (PCA) clearly separated the biopsies along the first dimension and distinguished the skin from patients with clinical positive elicitation reaction from patients with negative elicitation reactions (score plot is seen in fig. 6.). The first two dimensions retained 22% and 11% of the variation in the dataset. Annotation analysis revealed that terms for biological processes related to immune response were overrepresented in the annotation genes defining the negative direction of the first PC axis.

The group of patients with psoriasis could not be distinguished from the healthy individuals regardless of positive or negative clinical elicitation reactivity in the PCA score plot.
To further investigate whether or not elicitation reactions were specifically down-regulated in patients with psoriasis, probe sets from psoriatic patients, as well as healthy individuals with a negative elicitation reaction, were selected for further analysis with t-test and subsequent correction for multiple testing with Bonferroni adjustment. When comparing healthy controls and patients with psoriasis, no significant difference in gene expression was found among the negative elicitation reactions.

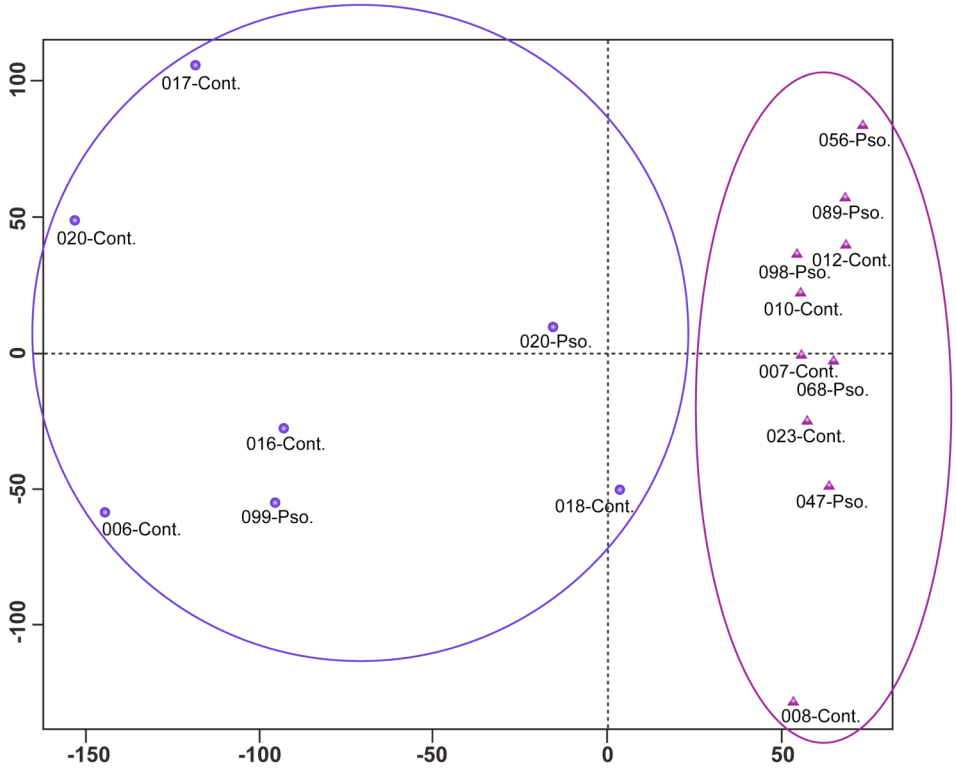


FIGURE 6: PCA plot, scores for the first and second axes. The projections of samples from subjects with a positive ○ and negative Δ elicitation reactions respectively are indicated by circles.

3.3.4. Methodological considerations, microarray

As in other experimental study-designs, conducting gene array experiments is accompanied with a line of decisions, subjective choices that can eventually influence the results.

We chose to use total RNA extracted from whole skin biopsies, which gave information from both epidermis and dermis. Epidermis and dermis have different cellular populations so the study would probably have been optimized by studying epidermis and dermis separately, as done in a recent microarray study of irritant skin reactions (67). The new Human Gene 1.0 ST arrays from Affymetrix were chosen. It has been validated with regards to probe-, exon- and gene-level reproducibility and found to be equivalent to the HGU 133 plus 2.0 arrays from Affymetrix used in previous studies of contact dermatitis (68). Choosing the computational approach to analysis of the microarray data is another point of subjective decision making, as the computational tools are rapidly evolving and there is no clear consensus as to the best method for revealing patterns of geneexpression.

We chose an unsupervised class discovery application, the Principal Component Analysis (PCA), to reveal patterns in the data without using sample classification. Another class discovery application, such as the widely used hierachical clustering algorithm, could also have been used but is unlikely to have affected the results. To bring about biological understanding of the findings of the PCA, a mechanistic analysis was applied with the Go Terms. In order to further investigate the possible difference between patients with psoriasis and controls, we used a class comparison application, the *t*-test. The issue of multiple testing is a concern with this statistical approach on array data. A stringent gene selection is required to avoid potential problems with false positive results, which is why a Bonferroni adjustment was applied. Using this correction, however, allows for the discarding of real differences in the gene expression. Interpretation of results is furthermore limited due to the small study sample.

3.3.5 Result specific discussion

Genome wide expression analysis on elicited skin has been performed in one other study using similar chip application on nickel-allergic individuals with results comparable to this study (69). A correspondence analysis clearly separated responders from non-responders with immune response being overrepresented in the annotation genes of the first axis. This suggests that the expression profiles are largely independent of the allergen involved. Similar to this result, no difference in gene expression profiles was found between non-responding, allergen challenged skin and skin where acetone control had been applied. This would indicate that non-respondence is not due to active down-regulation on site; however, interpretation of microarray data should be done cautiously. The gene expression analysis was done in order to generate hypothesis on the mechanisms behind the impaired contact allergic response in patients with psoriasis.

Even subtle changes in the laboratory protocol, parameters for slide scanning, image processing, data normalisation and the choice of analysis tools can contribute to a different outcome (70). The microarray analysis is, however, a robust analysis and only small and insignificant differences could have been overlooked in the study.

The results of the immunohistochemical staining of positive elicitation reactions are in line with other studies of human elicitation reactions. A mixed cell infiltrate dominated by CD8+ cells, believed to be the main effector cell and with FOXP3+ regulatory cells to counterbalance the reaction, in order to minimise collateral damage and eventually terminate the reaction (71, 72). CD4+ cells were found in the negative elicitation reactions. This is in line with findings by Clark et al. who showed that the stable skin under non-inflammatory conditions is harbouring T cells with the vast majority being CD4+ cells (73). The same group has demonstrated FOXP3+ in healthy skin and suggests that 10% of skin resident T cells are regulatory FOXP3+. The cells were visualised with immunoflouresence technique (74). We found a limited number of FOXP3+ cells using immunohistochemistry. This is in accord with other studies using this technique (75, 76). We did not find a higher number of FOXP3+ cells in the challenged sites of non-responders than in control sites; this indicates that no active down-regulation is taking place. Furthermore, we did not find any difference in the cell infiltrate among healthy controls and patients with psoriasis.

These results are in agreement with the findings of the microarray study and call for an alternative explanation of non-respondence in patients with psoriasis than differences in down-regulatory mechanisms.

4. GENERAL DISCUSSION

Contact allergy versus autoimmune disease - a true causal association?
An inverse association between contact allergy and psoriasis was found in the epidemiological studies. In the sensitization study psoriasis was found to affect the development of contact allergy. Using the methodological framework of Sir Bradford Hill's criteria for causation (77) on these studies, the association is likely to be a true causal association; Strength - The association is strong with relatively large odds ratios. Consistency - The result has been reproduced in an experimental study and two conceptually different epidemiological studies, studying different populations. Analogy - A similar inverse relation between contact allergy and other autoimmune diseases diabetes type 1, Crohn´s disease and rheumatoid arthritis has been found in previous studies (54-56). Coherence - The result does not conflict with what is known about the natural history of the diseases. Biological plausibility - It is biologically credible, that patients with psoriasis have an immunological milieu that can interfere with the development of another inflammatory disease, namely contact allergy. Temporality – In the sensitization study psoriasis is preceding contact allergy. Experiment – The association is shown in an experimental sensitization study. Only two criteria are not meet, that of specificity and biological gradient. The criterion of specificity is not possible to meet in this context as psoriasis cannot be added and removed as wanted. If the study group in the sensitization study had been larger, the effect of severity of psoriasis could have been estimated and a possible biological gradient established; however, seven out of nine of Bradford Hill's criteria are met and the found association likely to be true.

The sensitization study was conducted to show how psoriasis is affecting the development of contact allergy, but the possibility that contact allergy influences the immune system,

and thereby reduces the risk of psoriasis, was not investigated. The diseases may be mutually exclusive; this is another interesting area of research.

Regulatory mechanisms in contact allergy

An abundance of chemicals in the environment can elicit contact allergy. Everybody encounters allergens daily but only some develop contact allergy. Differences in exposures, as seen in occupational contact allergy can only account for some and it is still largely unknown why some individuals develop contact allergy to chemicals in the environment and others don't. The mechanisms behind this are only beginning to unravel. Are healthy hapten-exposed individuals non-allergic due to effective regulatory mechanisms in the sensitization phase, in the elicitation phase or in both phases?

A methodological problem in interpreting mechanistic events in human sensitization studies is the inability to distinguish these two phases, as the sensitization phase is defined by the outcome of elicitation. With a positive elicitation reaction it is evident that sensitization has preceded; however, with a negative elicitation reaction it is unknown if naïve T cells have not been primed in the sensitization phase, or the effector T cells are present but not activated or counterbalanced by regulatory T cells. Evaluating the sensitization phase is difficult in humans and knowledge on this phase stems primarily from murine studies.

At least two studies have given evidence to the idea that healthy individuals are actively kept non-allergic due to specific regulatory T cells acting during the elicitation phase. In a study by Cavani et al., CD4+CD25+ regulatory T cells were isolated from nickel challenged skin of healthy individuals (22) and Moed et al isolated CD4+CD25+ regulatory T cells from PBMC of healthy individuals (23). In both studies the isolated regulatory T cells were capable of suppressing proliferation of nickel specific effector CD4+CD25- T cells from nickel-allergic individuals in vitro.

In our study, no signs of deregulated genes were found in the challenge sites of non-responders neither in the psoriatic nor the healthy group. FOXP3 regulatory T cells were found in equal amount in the challenge sites of non-responders as in vehicle challenged

healthy skin; this indicates that non-respondence was not due to an active down-regulation through regulatory T cells in the skin, as seen in non-allergic subjects following nickel exposure. DPCP is an allergen for experimental use that is not found in the environment contrary to nickel, which has a high occurrence in the environment. It is possible that the regulatory T cells found in the nickel studies are dependent upon repeated low exposures and hence cannot be found for other less prevalent allergens, this remains to be further investigated.
Regulatory T cells have been found dysfunctional in suppressing auto-antigen specific effector T cells in various autoimmune diseases (42-45), but their regulation of environmental antigens were not investigated in these studies.

Psoriasis an autoimmune disease
In the sensitization study both psoriasis and diabetes type I was shown to affect the ability to develop contact allergy. This finding is in line with our epidemiological study and other recent epidemiological studies, showing an inverse relation between contact allergy and the autoimmune diseases; Rheumatoid arthritis, Crohns disease and diabetes type I (54-56). These autoimmune diseases, although clinically very different, appear in clusters and share many features including susceptibility loci and immunological pathways (14-16).
It seems likely, theoretically, that the autoimmune diseases share an immunological milieu that can interfere with the development/expression of contact allergy.
The importance of the Th1 cell in contact allergy is undisputed. Although IL-17 and Th17 cells have been demonstrated in allergic contact dermatitis in some studies, it is not believed to play a dominant role (78-80) hence the evidence of Th17 cell dominance in autoimmune diseases sets apart the autoimmune diseases from contact allergy and gives rise to a possible explanation of the found inverse relation.

It could be hypothesized that the highly Th17 directed cytokine milieu in patients with autoimmune diseases is interfering with the mounting of a contact allergic response.
The cytokine milieu is affecting the immune response in various ways: 1: Cytokines have been demonstrated to enhance differentiation of one T helper subset, in addition to inhibit the differentiation of other T helper subsets, thus favouring a specific immune response (81). In this way the Th17 polarized cytokine milieu in autoimmune diseases could hinder

the differentiation of naïve T cells to become effector or memory T cells necessary for the contact allergic immunological reaction. 2: Cytokines are suggested to regulate the function of regulatory T cells (82). Interestingly, a Th17 related cytokine IL-21 has been shown to render human CD4+CD25-, but not CD8+ T cells, resistant to the suppressive effects of regulatory T cells (83) and to induce apoptosis of antigen-specific CD8+ T cells (84). 3: Cytokines is believed to control APC function, which is pivotal for the contact allergic response. Brandt et al. demonstrated in a murine study, that short-time incubation of in vitro generated DC with IL-21 significantly reduced their potential to induce an antigen-specific CD8+ T cell proliferation (85, 86).

In a study by Cumberbatch et al., the uninvolved skin of psoriatic patients were associated with a normal amount of Langerhans cells (LC) that, however, displayed considerable impairment of mobilization. The authors hypothesised that this could be due to disease progression characterized by systemic changes that affect LC function (87). The systemic changes could very well be due to a Th17 skewed milieu found not only in patients with psoriasis but also in patients with other autoimmune diseases.

The found inverse relation between contact allergy and psoriasis is undoubtedly complex and not easily explained, but an immunological mechanistic understanding lie ahead when further research continue to unravel the pathogenesis of the two inflammatory diseases.

5. CONCLUSION AND PERSPECTIVES

The conclusions to be drawn from this phD thesis regarding contact allergy and psoriasis are:

I. Psoriasis and contact allergy are inversely related.
II. Patients with psoriasis have a reduced sensitization ratio towards DPCP compared with healthy controls.
III. The reduced sensitization ratio in patients with psoriasis is not due to down-regulatory mechanisms in the elicitation phase.

The finding of an inverse relation between contact allergy and psoriasis/autoimmune diseases is not only intriguing, it is of clinical importance. Research on finding new treatments for inflammatory diseases are today focused on molecular mechanisms in the inflammatory system, hence intensive knowledge on the complexity of these mechanisms, and how a disease process can affect the cause of another, are needed in order to avoid harmful side effects.

Research on the biological mechanisms behind the inverse relation between contact allergy and psoriasis can contribute to a more thorough understanding of the pathogenesis of both diseases and thereby, hopefully, lead to new, better and safer treatments.

6. SUMMERY

6.1 Summery in English

Allergic contact dermatitis (ACD) and psoriasis are the two most prevalent skin diseases in the western world. ACD is the clinical manifestation of contact allergy. Contact allergy and psoriasis are both due to inflammatory mechanisms involving the innate and adaptive immune system. Psoriasis is conceived to be an autoimmune disease. Recent studies have suggested an inverse relation between contact allergy and autoimmune diseases. The association between contact allergy and psoriasis could reveal mechanistic insights into both inflammatory processes.

The overall aim of this PhD study was to investigate the association between contact allergy and autoimmune disease, with focus on psoriasis. The work was done in three study parts. Part I Epidemiological studies. Part II Sensitization study and Part III Experimental studies.

In part I the association between contact allergy and psoriasis was investigated in two conceptually different epidemiological studies, a patient register-based study and a population-based study. A significant inverse relation between contact allergy and psoriasis was found in both studies with odds ratio for a person with a psoriasis diagnosis of having a positive patch-test of 0. 58 (CI 95% 0. 49 - 0.68) and 0.64 (CI 95% 0.42 - 0.98) respectively.

Part II was undertaken in order to investigate the potential of hapten sensitization and reactivity to challenge in patients with autoimmune disease. In a sensitization study using the strong hapten Diphenylcyclopropenone (DPCP) five groups of patients were induced and challenged: psoriasis, diabetes type I, mono-allergy, poly-allergy and healthy controls. Sensitisation ratios of patients with psoriasis and diabetes type I were significantly lower than healthy controls, with odds ratios of 0.18 (CI 95% 0. 039 - 0.85) P=0.031 and 0.74 (95% CI 0.548 – 1.008) P= 0.056 respectively. Sensitisation ratios of mono-allergic, poly-allergic and healthy controls were equal.

A trend towards a lower reactivity in the psoriatic group and a higher reactivity in the poly-allergic group was found. A significantly lower challenge threshold was found in the poly-allergic group compared to the healthy and mono-allergic group, with $p=0.005$ and $p=0.037$ respectively.

In part III mechanisms behind the reduced reactivity seen in patients with psoriasis were investigated. Using biopsies from challenge sites, the possibility of down-regulatory events in the elicitation phase was investigated. No regulatory FoxP3+ T cells were found in challenge sites from non-responders on immunohistochemical staining and no significant changes in mRNA expression were found with microarray analysis. This indicates that non-responding was not due to an active down-regulation at the challenge site in patients with psoriasis or healthy controls.

In conclusion the studies have contributed with evidence to support the theory that autoimmune diseases are inversely related to contact allergy. The studies have demonstrated that patients with autoimmune diseases have a reduced sensitization potential and suggest that this is not due to down-regulatory events in the elicitation phase.

6.2 Summery in Danish

Allergisk kontakt eksem og psoriasis er de to mest prævalente hud sygdomme i den vestlige verden. Allergisk kontakt eksem er den kliniske manifestation af kontakt allergi. Kontakt allergi og psoriasis er begge forårsagede af inflammatoriske mekanismer, der involvere både det innate og det adaptive immune system. Psoriasis opfattes desuden som en autoimmun sygdom. Nylige studier har peget på en invers relation mellem kontakt allergi og autoimmune sygdomme. Kendskab til associationen mellem kontakt allergi og autoimmune sygdomme som psoriasis vil kunne give indblik i sygdoms specifikke mekanismer for begge inflammatoriske processer.

Det overordnede mål med dette phD studie var at undersøge forholdet mellem kontakt allergi og autoimmune sygdomme med fokus på psoriasis. Arbejdet blev gennemført i tre studie dele. Del I epidemiologiske studier, del II Sensitiserings studier og del III eksperimentelle studier.

I del I blev forholdet mellem kontakt allergi og psoriasis undersøgt i to konceptuelt forskellige epidemiologiske studier; et patient register-baseret studie og et befolknings studie. En signifikant invers relation mellem kontakt allergi og psoriasis blev fundet i begge studier, med odds ratio for at en person med en psoriasis diagnose havde en positive lappeprøve på 0. 58 (CI 95 % 0, 49 - 0,68) and 0,64 (CI 95 % 0,42 – 0,98) respektivt.

Del II blev gennemført for at undersøge potentialet for hapten sensitisering og reaktion på challenge hos patienter med autoimmune sygdom. I et sensitiserings studie med anvendelse af det stærke hapten Diphenylcyclopropenone (DPCP) gennemgik fem grupper af patienter induktion og challenge: psoriasis, diabetes type I, mono-allergi, poly-allergi og raske kontroller.
Sensibiliserings ratioer for patienter med psoriasis og diabetes type I var signifikant lavere end raske kontroller, med odds ratioer på 0,18 (CI 95 % 0, 039 - 0,85) P=0,031 og 0,74 (95 % CI 0,548 – 1,008) P= 0,056 respektive. Sensibiliserings ratioer for mono-allergikere, poly-allergikere og raske kontroller var ens.

Der fandtes en trend mod lavere reaktivitet i psoriasis gruppen og højere reaktivitet i gruppen af polly-allergikere, der desuden fandtes at have en signifikant lavere challenge tærskel sammenlignet med den raske kontrol gruppe og gruppen af mono-allergikere p=0,005 and p=0,037 respektivt.

I del III undersøgtes mekanismer bag den fundne lavere reaktivitet hos psoriasis patienter. Hud biopsier fra challenge reaktionerne blev undersøgt for mulige ned-regulerende forhold i elicitationsfasen. Ved immunhistokemisk farvning fandtes der ingen regulatoriske FOXP3+ T celler i challenge reaktionerne fra deltagere med negative reaktioner ej heller signifikante ændringer i mRNA ekspression med microarray analyse. Dette indikerer, at negativ reaktion ikke var grundet en aktiv nedregulering i challenge områderne hos patietner med psoriasis eller raske kontroller.

Studierne bidrager samlet set med evidens til at støtte teorien om at kontakt allergi og autoimmune sygdomme er invers relaterede og demonstrere at patienter med autoimmune sygdomme har et nedsat potentiale for udvikling af kontakt allergi. Flere studier er nødvendige for at kortlægge årsagen til denne sammenhæng.

7. REFERENCES

1. Thyssen JP, Linneberg A, Menné T et al. The epidemiology of contact allergy in the general population--prevalence and main findings. Contact Dermatitis. 2007; 57:287-99
2. Mortz CG, Lauritsen JM, Bindslev-Jensen C, Andersen KE. Contact allergy and allergic contact dermatitis in adolescents: prevalence measures and associations. The Odense Adolescence Cohort Study on Atopic Diseases and Dermatitis (TOACS). Acta Derm Venereol. 2002; 82:352-8.
3. Nielsen NH, Menné T. Allergic contact sensitization in an unselected Danish population. The Glostrup Allergy Study, Denmark Acta Derm Venereol. 1992; 72:456-60
4. Bolognia, Jorizzo, Rapini. Dermatology Volume 1. Mosby s.227-239
5. Brasch J, Henseler T, Aberer W et al. Reroducability of patch tests. A multicenter study of synchronous left-versus right-sided patch tests by German Contact Dermatitis Research Group. J Am Acad Dermatol 1994; 31: 584-91.
6. Brandrup F, Green A. The prevalence of psoriasis in Denmark. Acta Derm Venereol. 1981; 61: 344-6.
7. Lomhold G. Prevalence of skin diseases in a population; a census study from the Faroe Islands. Dan Med Bull. 1964; 11:1-7.
8. Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med. 2009 30;361:496-509
9. Bolognia et al. Dermatology. Volume 1. Mosby S.136-137.
10. Brandrup, F et al.Psoriasis in monozygotic twins: variations in expression in individuals with identical genetic constitution. Acta Dermatol. Venereol. 1982; 62, 229–236
11. Duffy, D.L et al.Psoriasis in Australian twins. J. Am. Acad. Dermatol. 1993; 29, 428–434
12. Bowcock, A.M. and Krueger, J.G.Getting under the skin: the immunogenetics of psoriasis. Nature Rev. Immunol. 2005; 5, 699–711
13. Nair, R.P. et al. Sequence and haplotype analysis of supports HLA-C as the psoriasis susceptibility1 gene. Am. J. Hum. Genet. 2005; 78, 827–851
14. Christophers E. Comorbidities in psoriasis. Clinics in Dermatology 2007; 25: 529–534
15. Wolf N, Quaranta M, Prescott NJ, Allen M, Smith R, Burden AD, Worthington J, Griffiths CEM, Mathew CG, Barker JN. Psoriasis is associated with pleiotropic susceptibility loci identified in type II diabetes and Crohn disease. J. Med. Genet. 2008; 45; 114-116
16. Langrish CL, Chen, Blumenschein WM. Et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005; 201:233-40
17. Rustemeyer T, Van Hoogstraten IMW, Von Blomberg BM, Scheper RJ. Mechanisms in Allergic Contact Dermatitis. In: Frosch PJ, Menné T, Lepoittevin J-P, editors. Contact Dermatitis. 4 th. Ed. Berlin Heidelberg: Springer; 2006. p.11-43

18. Vocanson M, Hennino A, Rozières A, Poyet G, Nicolas JF. Effector and regulatory mechanisms in allergic contact dermatitis. Allergy. 2009; 64:1699-714.
19. Sebastiani S, Albanesi C, Nasorri F, Girolomoni G, Cavani A. Nickel-Speci®c CD4+ and CD8+ T Cells Display Distinct Migratory Responses to Chemokines Produced During Allergic Contact Dermatitis J Invest Dermatol 2002, 118:1052-1058
20. Sebastiani S, Allavena P, Albanesi C, Nasorri F, Bianchi G, Traidl C, Sozzani S, Girolomoni G, Cavani A. Function in CD4 + T Lymphocytes Chemokine Receptor Expression and with Regulatory Activity J. Immunol. 2001;166;996-1002
21. Ring S et al. CD4+CD25+ regulatory T cells supress contact hypersensitivity reactions by blocking influx of effector T cells into inflamed tissue. Eur J Immunology 2006; 36:2981-2992.
22. Cavani A, Nasorri F, Ottaviani C, Sebastiani S, De Pità O, Girolomoni G. Human CD25+ Regulatory T Cells Maintain Immune Tolerance to Nickel in Healthy, Nonallergic Individuals. J Immunol. 2003 1; 171:5760-8.
23. Moed H, von Blomberg BM, Bruynzeel DP, Scheper RJ, Gibbs S, Rustemeyer T. Regulation of nickel-induced T-cell responsiveness by CD4+CD25+ cells in contact allergic patients and healthy individuals. Contact Dermatitis. 2005; 53(2):71-4.
24. Brian J. Nickoloff, Jian-Zhong Qin, Frank O. Nestle. Immunopathogenesis of Psoriasis Clinic Rev Allerg Immunol 2007; 33:45–5635.
25. Wrone-Smith, T., and B. J. Nickoloff. . Dermal injection of immunocytes induces psoriasis. J. Clin. Invest. 1996; 98:1878.
26. Prinz J. C., B. Gross, S. Vollmer, P. Trommler, I. Strobel, M. Meurer, and G. Plewig. T cell clones from psoriasis skin lesions can promote keratinocyte proliferation in vitro via secreted products. Eur. J. Immunol. 1994; 24:593.
27. Bata-Csorgo Z, Hammerberg C, Voorhees JJ, Cooper KD. Kinetics and regulation of human keratinocyte stem cell growth in short-term primary ex vivo culture: cooperative growth factors from psoriatic lesional T lymphocytes stimulate proliferation among psoriatic uninvolved, but not normal, stem keratinocytes. J. Clin. Invest. 1995. 95:317.
28. Strange P, Cooper KD, Hansen ER, Fisher G, Larsen JK, Fox D, Krag C, Voorhees JJ, Baadsgaard O.T-lymphocyte clones initiated from lesional psoriatic skin release growth factors that induce keratinocyte proliferation. J Invest Dermatol. 1993; 101:695-700.
29. Steinman L.A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell–mediated tissue damage. Nature Med. 2007; 13:139-45.
30. Pène J, Chevalier S, Preisser L, Vénéreau E, Guilleux MH, Ghannam S, Molès JP, Danger Y, Ravon E, Lesaux S, Yssel H, Gascan H. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. J Immunol. 2008 1; 180:7423-30.
31. Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, Bowman EP, Krueger JG. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol. 2008; 128:1 207-11
32. Kagami S, Rizzo HL, Lee JJ, Koguchi Y, Blauvelt A. Circulating Th17, Th22, and Th1 cells are increased in psoriasis. J Invest Dermatol. 2010; 130:1373-83.

33. Wilson NJ, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol 2007*;* 8:950–957.
34. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, McClanahan TK, O'Shea JJ, Cua DJ. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat Immunol. 2009; 10:314-24.
35. Papp KA, Langley RG, Lebwohl M, Krueger GG, Szapary P, Yeilding N, Guzzo C, Hsu MC, Wang Y, Li S, Dooley LT, Reich K; PHOENIX 2 study investigators. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2).Lancet. 2008; 371:1675-84.
36. Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, Goldgar D, Gudjonsson JE, Li Y, Tejasvi T, Feng BJ, Ruether A, Schreiber S, Weichenthal M, Gladman D, Rahman P, Schrodi SJ, Prahalad S, Guthery SL, Fischer J, Liao W, Kwok PY, Menter A, Lathrop GM, Wise CA, Begovich AB, Voorhees JJ, Elder JT, Krueger GG, Bowcock AM, Abecasis GR; Collaborative Association Study of Psoriasis. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathway. Nat Genet. 2009; 41:199-204..
37. Capon F, Di Meglio P, Szaub J et al.Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. Hum Genet 2007; 122: 201–6.
38. Cargill M, Schrodi SJ, Chang M et al. A large scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am. J Hum Genet 2007; 80:273–90.
39. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self tolerance and autoimmune disease. Immunol Rev 2006; 212:8–27
40. Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: forkhead box protein 3 mutations and lack of regulatory T cells.J Allergy Clin Immunol. 2007; 120:744-50.
41. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001; 27:20-1.
42. Sugiyama H, Gyulai R, Toichi E, Garaczi E, Shimada S, Stevens SR, McCormick TS, Cooper KD. Dysfunctional blood and target tissue CD4+CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. J Immunol. 2005; 1; 174:164-73.
43. Viglietta, V., C. Baecher-Allan, H. L. Weiner, and D. A. Hafler. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J. Exp. Med. 2004; 199:971.
44. Kriegel, M. A., T. Lohmann, C. Gabler, N. Blank, J. R. Kalden, and H. M. Lorenz. Defective suppressor function of human CD4+ CD25+regulatory T cells in autoimmune polyglandular syndrome type II. J. Exp. Med.2004; 199:1285.

45. Lawson JM, Tremble J, Dayan C, Beyan H, Leslie RDG, Peakman M, Tree TIM. Increased resistance to CD4+CD25hi regulatory T cell-mediated suppression in patients with type 1 diabetes.Clin Exp. Immunology. 2008
46. Henseler T, Christophers E. Disease concomitance in psoriasis. J Am Acad Dermatol. 1995; 32:982-6
47. Fedler R, Strömer K. Nickel sensitivity in atopics, psoriatics and healthy subjects. Contact Dermatitis. 1993; 29: 65-9
48. Barile M, Cozzani E, Anonide A et al. Is contact allergy rare in psoriatics? Contact Dermatitis.1996; 35:113-4
49. Jovanović M, Boža P, Karadaglić D et al. Contact Sensitivity in Patients with Psoriasis in Vojvodina. Int Arch Allergy Immunol. 2008; 148: 311-320.
50. Heule F, Tahapary GJ, Bello CR et al. Delayed-type hypersensitivity to contact allergens in psoriasis. A clinical evaluation. Contact Dermatitis.1998; 38:78-82.
51. Moss C, Friedmann PS, Shuster S. Impaired contact hypersensitivity in untreated psoriasis and the effects of photochemotherapy and dithranol/UV-B. Br J. Dermatol. 1981; 105: 503-8.
52. Obalek S, Haftek M, Slinski W. Immunological studies in psoriasis. The quantitative evaluation of cell-mediated immunity in patients with psoriasis by experimental sensitization to 2, 4-dinitrochlorobenzene. Dermatologica. 1977; 155:13-25
53. Epstein WL, Maibach HI. Immunological competence of patients with psoriasis receiving cytotoxic drug therapy. Arch Dermatol. 1965; 91:599-606.
54. Engkilde, K, Menné, T. and Johansen, J. D. PB.39 Inverse association between rheumatoid arthritis and allergic contact dermatitis. Contact Dermatitis 58 (Suppl. 1.), 68-69. 27-5-2008. Ref Type: Abstract
55. Engkilde K, Menné T, Johansen JD. Inflammatory bowel disease in relation to contact allergy: a patient-based study. Scand J Gastroenterology. 2007; 42: 572-6.
56. Engkilde K, Menné T, Johansen JD. Inverse relationship between allergic contact dermatitis and type 1 diabetes mellitus: a retrospective clinic-based study. Diabetologica 2006; 49: 644-7.
57. Epstein WL, Jessar RA.Contacttype delayed hypersensitivity in patients with rheumatoid arthritis. Arthritis Rheum 1959;2:178-81
58. Nickelsen TN. Data validity and coverage in the Danish National Health Registry. A literature review. Ugeskr. Laeger. 2001; 164:33-7
59. Jagou M, Bastuji-Garin S, Bourdon-Lanoy E et al. Poor agreement between self-reported and dermatologists' diagnoses for five common dermatoses. 2006;155:1006-12
60. Henseler T, Christophers E. Psoriasis of early and late onset: Characterization of two types of psoriasis vulgaris. Journal of the American Academy of Dermatology.1985; 450-456
61. Friedmann PS, Moss C, Shuster S, Simpson S. Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. Clin. Exp. Immunol. 1983; 53: 709-715.

62. Cooper K.D, Oberhelman L, Hamilton T.A, Baadsgaard O, Terhune M et al. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: Relationship to dose, $CD1a^{-}$ DR^{+} epidermal macrophage induction, and Langerhans cell depletion. Proc.Natl.Acad.Sci 1992; 89; 8497-8501
63. Skov L, Hansen H, Barker JN, Simon JC, Baadsgaard O. Contrasting effects of ultraviolet-A and ultraviolet-B exposure on induction of contact sensitivity in human skin.Clin Exp Immunol. 1997; 107:585-8.
64. Nassif A, Chan SC, Storrs FJ, et al. Abnormal skin irritivity in atopic dermatitis and in atopy without dermatitis. Arch Dermatol.1994; 130: 1402-7
65. Moss C, Friedmann PS, Shuster S, Simpson JM. Susceptibility and amplification of sensitivity in contact dermatitis. Clin. Exp. Immunol. 1985; 61: 232-241.
66. Bacchetta R, Gambineri E, Roncarolo MG. Role of regulatory T cells and FOXP3 in human diseases.J Allergy Clin Immunol. 2007; 120:227-35
67. Clemmensen A, Andersen KE, Clemmensen O, Tan Q, Petersen TK, Kruse TA, Thomassen M. Genome-Wide Expression Analysis of Human In Vivo Irritated Epidermis: Differential Profiles Induced by Sodium Lauryl Sulfate and Nonanoic Acid. J Invest Dermatol. 2010; 29.
68. Robinson MD, Speed TP. A comparison of Affymetrix gene expression arrays.BMC Bioinformatics. 2007; 15; 8:449.
69. Pedersen MB, Skov L, Menné T, Johansen JD, Olsen J. Gene expression time course in the human skin during elicitation of allergic contact dermatitis. J Invest Dermatol. 2007; 127:2585-95.
70. Quackenbush J. Computational analysis of microarray data. Nat Rev Genet. 2001 ;2:418-27
71. Cavani A, Mei D, Guerra E, Corinti S, Giani M, Pirrotta L, Puddu P, Girolomoni G. Patients with allergic contact dermatitis to nickel and nonallergic individuals display different nickel-specific T cell responses. Evidence for the presence of effector CD8+ and regulatory CD4+ T cells. J invest Dermatol 1998;111:621-8
72. Bour H, Peyron E, Gaucherand M, Garrigue JL, Desvignes C, Kaiserlian D, Revillard JP, Nicolas JF. Major histocompatibility complex class I-restricted CD8+ T cells and class II-restricted CD4+ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene.Eur J Immunol. 1995; 25:3006-10.
73. Clark RA, Chong B, Mirchandani N, Brinster NK, Yamanaka K, Dowgiert RK, Kupper TS. The Vast Majority of CLA+ T Cells Are Resident in Normal Skin. J. Immunol. 2006; 176; 4431-4439
74. Clark RA, Kupper TS. IL-15 and dermal fibroblasts induce proliferation of natural regulatory T cells isolated from human skin Blood. 2007; 1; 109: 194–202.
75. Marzia Caproni Emiliano Antiga, Daniele Torchia, Walter Volpi, Emanuela Barletta, Gianni Gitti, Enrico De Campora, Paolo Fabbri FoxP3-expressing T regulatory cells in atopic dermatitis lesions Allergy Asthma Proc 2007; 28:525–528
76. Fujimura T, R. Okuyama, Y. Ito and S. Aiba Profiles of Foxp3+ regulatory T cells in eczematous dermatitis, psoriasis vulgaris and mycosis fungoides British Journal of Dermatology 2008; 158:1256–1263
77. Hill AB. Principles of medical statistics. 8th ed. London. The Lancet; 1966.

78. Zhao Y, Balato A, Fishelevich R, Chapoval A, Mann DL, Gaspari AA. Th17/Tc17 infiltration and associated cytokine gene expression in elicitation phase of allergic contact dermatitis. Br J Dermatol. 2009; 161:1301-6.
79. He D, Wu L, Kim HK, Li H, Elmets CA, Xu H. IL-17 and IFN-gamma mediate the elicitation of contact hypersensitivity responses by different mechanisms and both are required for optimal responses.J Immunol. 2009 15**;** 183:1463-70.
80. Larsen JM, Bonefeld CM, Poulsen SS, Geisler C, Skov L. IL-23 and T (H) 17-mediated inflammation in human allergic contact dermatitis.J Allergy Clin Immunol. 2009; 123:486-92.
81. Rautajoki KJ, Kylamk, Niemi, Raghav SK, Rao K, Lahesmaa R. An insight into molecular mechanisms of human T helper cell differentiation Annals of Medicine. 2008; 40: 322-335
82. Goodman WA, Levine AD, Massari JV, Sugiyama H, McCormick TS, Cooper KD. IL-6 signaling in psoriasis prevents immune suppression by regulatory T cells. J Immunol. 2009 1;183:3170-6.
83. Peluso, I. et al. IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes. J. Immunol. 2007; 178, 732–739
84. Brianne R. Barker, Jenny G. Parvani, Meyer D, Adam S. Hey, Skak K, Norman L. Letvin. IL-21 Induces Apoptosis of Antigen-Specific CD8+ T Lymphocytes The Journal of Immunology, 2007, 179: 3596–3603.
85. Brandt K, Bulfone-Paus S, Jenckel A, Foster DC, Paus R, Rückert R. Interleukin-21 inhibits dendritic cell-mediated T cell activation and induction of contact hypersensitivity in vivo.J Invest Dermatol. 2003; 121:1379-82.
86. Brandt K, Bulfone-Paus S, Foster DC, Rückert R. Interleukin-21 inhibits dendritic cell activation and maturation. Blood. 2003 1; 102:4090-8.
87. Cumberbatch M, Singh M, Bearman RJ et al. Impaired Langerhans cell migration in psoriasis. J. Exp. Med 2006; 203: 953-60.

8. MANUSCRIPTS

Inverse relationship between contact allergy and psoriasis: results from a patient- and a population-based study

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Summary

Background An inverse association between contact allergy and autoimmune diseases has been suggested. Psoriasis is an autoimmune disease and it has been debated whether contact allergy is less prevalent among patients with psoriasis. Previous studies have shown conflicting results.

Objectives To examine a possible association between contact allergy and psoriasis in two conceptually different epidemiological studies.

Patients and methods Two study populations were included: (i) a clinic-based register linkage study population, achieved by record linking information from the Danish National Hospital Registry identifying patients with psoriasis with information on contact allergy from a comprehensive patch test database of 15 641 patients; and (ii) a population-based cross-sectional study population organized in 1990, 1998 and 2006 and obtained by random samples from the Danish Central Personal Register. Information was obtained by questionnaire and patch testing of 4989 subjects.

Results An inverse association was found between a psoriasis diagnosis and a positive patch test in both studies. The odds ratio for a person with a psoriasis diagnosis of having a positive patch test was, adjusted for sex and age, 0·58 [95% confidence interval (CI) 0·49–0·68] and 0·64 (95% CI 0·42–0·98), respectively, in the two studies.

Conclusions The finding of an inverse association between psoriasis and contact allergy may express opposite immunological mechanisms and calls for additional research in this field.

Contact allergy and psoriasis are prevalent inflammatory skin diseases affecting 15–20% and 2–3%, respectively, of the general population.[1,2] It has been debated whether contact allergy is less prevalent among patients with psoriasis. One epidemiological study found that contact allergy was three times less frequent among patients with psoriasis than among patients with a nonpsoriatic skin disease.[3] The overall prevalence of contact allergy in patients with psoriasis has been investigated previously and revealed that around 20–25% of patients with psoriasis had contact allergy;[4–6] however, in one study, the frequency was as high as 68%.[7] The conflicting results show that there are a number of difficulties in conducting such studies. These difficulties can be attributed to insufficient sample size, inaccuracy of the psoriasis diagnosis, differences in patch testing and reading of results, alongside confounding factors such as ultraviolet (UV) exposure and systemic treatment. In an experimental study with standardized parameters and limited potential confounding factors, Moss *et al.*[8] found that untreated patients with psoriasis were significantly less responsive to sensitization with dinitrochlorobenzene (DNCB) than healthy controls. Comparable results were found in a similar study by Obalek *et al.*,[9] who furthermore showed that the impairment of reactivity was inversely related to the activity of psoriasis. The low sensitivity could relate to accelerated epidermal turnover as seen in psoriasis, or to functional alterations in the immune status in psoriasis. Psoriasis is generally considered to be an autoimmune disease, which shares many features, including pathological pathways and therapeutic targets, with other inflammatory autoimmune diseases such as rheumatoid arthritis and Crohn disease.[10,11]

An inverse association between autoimmune diseases and contact allergy has previously been identified.[12–14] This

finding suggests that a shared autoimmune immunological milieu may interfere with the mounting of a contact allergic response. This study investigated the association between contact allergy and psoriasis by linking information from two large patient databases and by using patch test and questionnaire data from the largest cross-sectional patch test study ever performed in the general population.

Patients and methods

Study populations

The study was based on two study populations.

Clinic-based register linkage study population

At the Department of Dermato-Allergology, Gentofte Hospital, University of Copenhagen, Denmark, all patch test results, along with the tested patients' demographic data, have been consecutively registered in a comprehensive, computerized database. Data for the current study was obtained from this database for the period 1985–2006.

The Danish National Hospital Registry (DNHR) holds information on all discharges from Danish hospitals since 1977; outpatients have been included in the register since 1 January 1995. The register includes information on discharge diagnoses, hospital, department and day of discharge for both in- and outpatients. Discharge diagnoses are classified in the DNHR according to a Danish version of the International Classification of Diseases (ICD). ICD8 was used from 1977 to 1993 and ICD10 from 1994 onwards. Psoriasis is registered with ICD8 codes 69609, 69610, 69619 and ICD10 code DL40.

Information from the patch test database and the DNHR were linked by means of the unique civil registry number given to all Danish citizens at birth.

Age was defined as the age at the first positive patch test; if there was no positive patch test, the age at the date of first patch testing was used. The patients were stratified into five age groups: 0–15, 16–30, 31–50, 51–70 and > 70 years.

Population-based cross-sectional study population

In 1990, 1998 and 2006, three consecutive cross-sectional studies were organized by the same investigators (T.M., N.H.N. and A.L.). Danish adults (with Danish citizenship and born in Denmark) living in one of the same 11 municipalities of the Copenhagen County were invited to participate in a general health examination including patch testing. Study populations were random samples obtained from the Danish Central Personal Register, Ministry of Internal Affairs. Participants completed a postal questionnaire asking the question: 'Has your doctor ever told you that you suffer from psoriasis?' The Ethical Committee of Copenhagen County approved all studies.

Between February 1990 and January 1991, 1112 (77·5%) of 1435 invited subjects aged 15–69 years participated in a health examination and 1056 (73·6%) were patch tested. Between January and November 1998, 482 (53·4%) of 902 invited subjects aged 15–41 years participated and of these 473 (52·4%) were also patch tested. Finally, from June 2006 to May 2008, 3471 (43·7%) of 7931 invited subjects aged 18–69 years accepted the invitation to a health examination and of these subjects 3460 (43·6%) were also patch tested. For practical reasons, persons below the age of 18 years were not invited in 2006 as an informed, written consent from the parents is now mandatory in Denmark. The characteristics of participants and nonparticipants in the 1990, 1998 and 2006 studies have previously been described in more detail.[15–17]

Patch testing

In the clinic-based register linkage study, all patients were patch tested with the European baseline series recommended at the time of testing. The European baseline series contains the most prevalent contact allergens in the environment for continental Europe. Patch testing is performed, according to international standards, on the upper back using Trolab allergens (Hermal, Reinbek, Germany) and Finn chambers for occlusion (Epitest, Tuusula, Finland). Occlusion time is 48 h and patches are read on days 2, 3 or 4 and 5 or 7 in accordance with international criteria from the International Contact Dermatitis Research Group (ICDRG).[18,19] A positive allergic reaction is defined as being at least grade 1+ according to ICDRG.

In the population-based cross-sectional studies, patch testing was performed by using panels 1 and 2 from the standardized, ready to apply, Thin-layer Rapid Use Epicutaneous (TRUE)-Test (supplied by ALK-Abelló A/S, Hørsholm, Denmark in 1998 and Mekos Laboratories, Hillerød, Denmark in 2006). Directions to apply the patch test panels to the upper back 2 days before examination were mailed along with the patch tests. Patch tests were read and photographed 1–1½ h after removal. The photos were reviewed by N.H.N. and T.M. in 1990, by N.H.N., T.M. and A.L. in 1998 and by T.M., N.H.N., A.L. and J.P.T. in 2008. This was done to ensure that the ICDRG criteria were used consistently over time. Contact sensitization was defined as a positive (at least grade 1+ according to ICDRG) patch test to at least one allergen or mixes of haptens). If the patch had no skin contact upon patch test reading, or if the subject had removed it prior to testing as a result of known contact sensitization, it was regarded as missing data.

Statistical analysis

In both studies, the association between contact allergy and psoriasis was explored by using a χ^2 test and by using logistic regression analyses. The outcome of patch testing was used as the dependent variable and psoriasis, sex and age as the independent variables. Results were expressed as odds ratios (OR) with 95% confidence intervals (CI). All data analysis was performed using SPSS version 12 (SPSS, Chicago, IL, U.S.A.).

Results

Clinic-based register linkage study

Between 1985 and 2006, 15 641 patients were patch tested at Gentofte Hospital, University of Copenhagen, Denmark. More females than males were tested in this period (63·7% vs. 36·3%). The age, defined as the age at the first positive patch test or the first patch test if all negative, spanned from 4 to 99 years, with a median of 47·0 years; 9463 patients had patch tests with a positive outcome (35·6%). There were more positive outcomes among females than males (41·1% vs. 26·0%).

Of the 15 641 patients 806 had an ICD code for psoriasis (ICD8 69609, 69610, 69619 or ICD10 DL40). An outline of the linked register data is shown in Table 1.

An inverse association was found between a psoriasis diagnosis and a positive patch test. The crude OR for a person with a psoriasis diagnosis of having a positive patch test was 0·58 (95% CI 0·49–0·68). The result did not change when adjustment for sex and age was applied (Table 2).

Population-based cross-sectional study

Between 1990 and 2006, 4989 (98·5%) of 5065 participants were patch tested; 616 (12·3%) of these had a positive patch test reaction to at least one allergen; 301 (6·1%) of 4989 respondents reported that their doctor had at some point told them that they suffered from psoriasis. By use of a logistic regression model we explored the association between psoriasis and contact allergy. Crude data analysis revealed an inverse association between self-reported psoriasis and a positive patch test reaction to at least one allergen with an odds ratio of 0·67 (95% CI 0·44–1·00). After adjustment for sex and age group the OR was 0·64 (95% CI 0·42–0·98) (Table 3).

Table 2 Results from the logistic regressions on the clinic-based study with contact allergy as the response variable and psoriasis as the independent variable

	P	Odds ratio	95% CI
Psoriasis	< 0·0001	0·581	0·494–0·684
Psoriasis adjusted for sex and age	< 0·0001	0·581	0·492–0·685

Table 3 Results from the logistic regressions on the population-based cross-sectional study with contact allergy as the response variable and psoriasis as the independent variable

	P	Odds ratio	95% CI
Psoriasis	0·049	0·67	0·44–1·00
Psoriasis adjusted for sex and age	0·038	0·64	0·42–0·98

Discussion

We found an almost identical and inverse association between psoriasis and contact allergy in two conceptually different studies. The association was significant for patients from a dermatological department as well as for subjects in the general population. Thus our data strongly support that these two skin disorders are inversely related. Reports on the relation between contact allergy and psoriasis from such epidemiological studies have never been presented before. It was only possible due to the collection of large patient populations and the unique identifier system in Denmark, which makes a linkage possible between such registers.

The association of psoriasis and contact allergy has previously been investigated using different approaches. Using a large database of dermatological patients, Henseler and Christophers investigated disease concomitance in psoriasis. Contact allergy was found to be three times less frequent in patients with psoriasis compared with a control group of patients with nonpsoriatic skin diseases.[3] The patients were not patch tested and it could be argued that some of the patients with psoriasis may have had undiagnosed contact allergy. Conflicting results have been presented in studies, in which patch testing of patients with psoriasis have been compared with control groups. Fedler and Strömer patch tested 552 adults, 88 of whom had psoriasis, with standard allergens and found that patients with psoriasis had significantly less nickel allergy than healthy controls but found no difference in the overall prevalence of contact allergy.[5] Barile *et al.* patch tested 305 patients with psoriasis and 96 patients with dermatological diseases, other than allergic contact dermatitis and psoriasis, and found no difference in responsiveness to patch testing between the groups.[4] In a recent study from Vojvodina, 56 patients with psoriasis and a control group was patch tested with the European baseline series.[6] No overall difference was found; however, male patients with psoriasis were found to have a lesser reaction than healthy males, although the difference was on the margin of significance. The different results reveal, as previously mentioned, difficulties in conducting such patient studies.

Table 1 Overview of contact allergy in the patch test register

	Negative patch test (n = 10 069)					Positive patch test (n = 5572)				
Age (years)	0–15	16–30	31–50	51–70	> 70	0–15	16–30	31–50	51–70	> 70
Psoriasis	10	79	206	234	77	0	18	73	81	28
Not psoriasis	213	2096	3129	2719	1306	51	941	2039	1622	719

In our study a few potential biases also need to be addressed. The identification of patients with psoriasis in the clinic-based study was based on data from the DNHR. This register has been validated in several studies for other diagnosis and found to be between 66% and 99% accurate.[20] Outpatients were not included in the DNHR until 1 January 1995. This could result in under-registration of patients with psoriasis, typically the milder cases. However, patients registered as outpatients from 1995 were all accounted for as inpatients. This supports the notion that being followed in a hospital department as an outpatient will lead to admission at some point; hence the lack of outpatient registrations in the first period is regarded as being of minor importance to the result, although the issue of sampling bias has to be considered. Mild cases of psoriasis are not included as they are not being followed in a hospital centre and therefore not identified in the registry. This, however, is not the case in the population-based study design in which all degrees of psoriasis severity are likely to be represented. The similar results from the two studies indicate that the found inverse relation is evident for all mild as well as severe cases of psoriasis.

In the population-based study, the diagnosis of psoriasis was obtained by using a questionnaire. The prevalence of psoriasis in the dataset was relatively high (6%), suggesting that most people with the diagnosis of psoriasis are accounted for. Dermatitis is a prevalent skin disorder associated with contact allergy. It is possible that some participants incorrectly reported psoriasis instead of dermatitis. However, this would tend to underestimate an inverse association between psoriasis and contact allergy. The prevalence of a positive patch test reaction to at least one allergen was 12·3%, which is relatively low compared with previous reports from the general population. It has been estimated that approximately 18–29% of positive patch test reactions to nickel are missed when patch test readings are performed on day 2 only and not also on day 4.[21] Patch test readings in the study were done on day 2, which may account for the low prevalence. However, this is not likely to have influenced the inverse association found between psoriasis and contact allergy. Patients with psoriasis are often treated with UV light or systemic immunosuppressant medication, which could suppress a positive reaction at the patch testing. However, these treatments are as standard discontinued at least 4 weeks before testing and are therefore unlikely to have interfered with the result in any of the two studies.

A difference in the pattern of referral to patch testing, for patients with psoriasis compared with that of chronic dermatitis patients, could result in a bias in the register-based study. If such a difference exists it would be assumed to be more restricted for patients with psoriasis than for patients with chronic dermatitis, which would lead to a negative rather than a positive association and so would make the found negative association even stronger.

A well-known epidemiological variation in psoriasis exists, with relatively high prevalences in Northern Europe.[22] The variation in contact allergy is not elucidated in a similar way and will depend on exposure patterns. Theoretically patients with psoriasis could have a specific pattern of exposure to allergens; however, the relative distribution of allergies in patients with psoriasis in our material does not differ from the rest of the patients, which argues against a different exposure pattern for patients with psoriasis.

Our studies do not indicate the direction of causality of the found inverse association. Unlike contact allergy psoriasis has a strong genetic background but is likely to appear between the age of 15 and 30 years.[23] This is the same age that onset of many contact allergies are seen, which makes it difficult to assess the causality in the found inverse relation. The possibility that contact allergy influences the immune system, and thereby reduces the risk of psoriasis, cannot be ruled out from these studies; however, in a study with sensitization Moss *et al.*[8] found untreated patients with psoriasis to be significantly less responsive to DNCB than healthy controls. Comparable results were found in a similar study by Obalek *et al.*,[9] who furthermore showed the impairment of reactivity to be inversely related to the activity of psoriasis.

These studies support the concept of psoriasis as an immunodysregulatory process that interferes with the mounting of an immune response to haptens. Some investigators have attributed the low incidence of contact allergy in patients with psoriasis to accelerated epidermal turnover in psoriasis; others believe it to be due to functional alterations in T cells in psoriasis.[9]

In previous studies, using the same clinic-based register study design, we have found inverse relations between contact allergy and the autoimmune diseases type 1 diabetes, Crohn disease and rheumatoid arthritis.[12–14] These autoimmune diseases have in epidemiological studies been shown to be associated with psoriasis more frequently than expected and to share genetic susceptibility loci.[10,11] Although the disorders represent separate entities they appear to follow overlapping pathogenic pathways. In the past 3 years the importance of T-helper (Th) 17 cells in the pathogenesis of organ-specific autoimmune inflammation, including psoriasis, has been demonstrated through multiple areas of research, as reviewed by Steinman[24] and the cytokine signature of Th17 cells interleukin (IL)-17, IL-23, IL-22, IL-6 and IL-21 has been found to play a key role in the pathogenesis of several autoimmune diseases.[25–33] Apart from this, alterations of dendritic cell, regulatory T cell and cytokine homeostasis have been implicated in various human autoimmune diseases.[34,35] We believe that an immunological explanation for the inverse relation between contact allergy and psoriasis is to be found in the immunological milieu shared between the autoimmune diseases. The Th17 dominance in autoimmune diseases is only one of many factors that could play a role. Contact allergy is traditionally thought to be a Th1-mediated inflammatory disease, but recently Th17 cells have also been found to play a role in allergic contact dermatitis,[36] demonstrating the complex immunological nature of the inflammatory diseases. An exact explanation of how the autoimmune milieu can interfere with the induction and/or elicitation phase of the T-effector cell-mediated immune response in contact allergy or vice versa remains to be further elucidated.

In conclusion this study demonstrates an inverse relation between psoriasis, an autoimmune disease, and contact allergy. The relation is undoubtedly complex and it seems unlikely that a single approach will be found to describe it. Expanding our understanding of the immunological complexity is of major importance when considering therapeutic targets and further studies in these fields are required.

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References

1 Thyssen JP, Linneberg A, Menné T *et al.* The epidemiology of contact allergy in the general population—prevalence and main findings. *Contact Dermatitis* 2007; **57**:287–99.
2 Brandrup F, Green A. The prevalence of psoriasis in Denmark. *Acta Derm Venereol* 1981; **61**:344–6.
3 Henseler T, Christophers E. Disease concomitance in psoriasis. *J Am Acad Dermatol* 1995; **32**:982–6.
4 Barile M, Cozzani E, Anonide A *et al.* Is contact allergy rare in psoriatics? *Contact Dermatitis* 1996; **35**:113–14.
5 Fedler R, Strömer K. Nickel sensitivity in atopics, psoriatics and healthy subjects. *Contact Dermatitis* 1993; **29**:65–9.
6 Jovanović M, Boža P, Karadaglić D *et al.* Contact sensitivity in patients with psoriasis in Vojvodina. *Int Arch Allergy Immunol* 2008; **148**:311–20.
7 Heule F, Tahapary GJ, Bello CR *et al.* Delayed-type hypersensitivity to contact allergens in psoriasis. A clinical evaluation. *Contact Dermatitis* 1998; **38**:78–82.
8 Moss C, Friedmann PS, Shuster S. Impaired contact hypersensitivity in untreated psoriasis and the effects of photochemotherapy and dithranol/UV-B. *Br J Dermatol* 1981; **105**:503–8.
9 Obalek S, Haftek M, Slinski W. Immunological studies in psoriasis. The quantitative evaluation of cell-mediated immunity in patients with psoriasis by experimental sensitization to 2,4-dinitrochlorobenzene. *Dermatologica* 1977; **155**:13–25.
10 Christophers E. Comorbidities in psoriasis. *Clin Dermatol* 2007; **25**:529–34.
11 Griffiths CEM, Mathew CG, Barker JN *et al.* Psoriasis is associated with pleiotropic susceptibility loci identified in type II diabetes and Crohn disease. *J Med Genet* 2008; **45**:114–16.
12 Engkilde K, Menné T, Johansen JD. Inverse association between rheumatoid arthritis and allergic contact dermatitis. *Contact Dermatitis* 2008; **58** (Suppl. 1):68–9.
13 Engkilde K, Menné T, Johansen JD. Inflammatory bowel disease in relation to contact allergy: a patient-based study. *Scand J Gastroenterol* 2007; **42**:572–6.
14 Engkilde K, Menné T, Johansen JD. Inverse relationship between allergic contact dermatitis and type 1 diabetes mellitus: a retrospective clinic-based study. *Diabetologica* 2006; **49**:644–7.
15 Nielsen NH, Dirksen A, Madsen F. Can subjects with a positive allergen skin test be selected by a short questionnaire? The Glostrup Allergy Study, Denmark. *Allergy* 1993; **48**:319–26.
16 Nielsen NH, Linneberg A, Menné T *et al.* Allergic contact sensitization in an adult Danish population: two cross-sectional surveys eight years apart (the Copenhagen Allergy Study). *Acta Derm Venereol* 2001; **81**:31–4.
17 Thyssen JP, Linneberg A, Menne T *et al.* The prevalence and morbidity of sensitization to fragrance mix I in the general population. *Br J Dermatol* 2009 Apr 24 [Epub ahead of print].
18 Wilkinson DS, Fregert S, Magnusson B *et al.* Terminology of contact dermatitis. *Acta Derm Venereol* 1970; **50**:287–92.
19 Wahlberg J, Lindberg M. Patch testing. In: *Contact Dermatitis* (Frosch PJ, Menné T, Lepoittevin J-P, eds), 4th edn. Berlin, Heidelberg and New York: Springer, 2006; 365–90.
20 Nickelsen TN. Data validity and coverage in the Danish National Health Registry. A literature review. *Ugeskr Laeger* 2001; **164**:33–7.
21 Thyssen JP, Jensen CS, Johansen JD *et al.* Results from additional nickel patch test readings in a sample of schoolgirls from the general population. *Contact Dermatitis* 2008; **59**:317–18.
22 McFadden JP, Baker BS, Powles AV, Fry L. Psoriasis and streptococci: the natural selection of psoriasis revisited. *Br J Dermatol* 2009; **160**:929–37.
23 Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. *J Am Acad Dermatol* 1985; **13**:450–6.
24 Steinman L. A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell-mediated tissue damage. *Nat Med* 2007; **13**:139–45.
25 Langrish CL, Chen Y, Blumenschein WM *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005; **201**:233–40.
26 Capon F, Di Meglio P, Szaub J *et al.* Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. *Hum Genet* 2007; **122**:201–6.
27 Cargill M, Schrodi SJ, Chang M *et al.* A large scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007; **80**:273–90.
28 Park H, Li Z, Yang XO *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; **6**:1133–41.
29 Komiyama Y, Nakae S, Matsuki T *et al.* IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol* 2006; **177**:566–73.
30 Nakae S, Nambu A, Sudo K *et al.* Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J Immunol* 2003; **171**:6173–7.
31 Chen Y, Langrish CL, McKenzie B *et al.* Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. *J Clin Invest* 2006; **116**:1317–26.
32 Zhou L, Ivanov II, Spolski R *et al.* IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 2007; **8**:967–74.
33 Korn T, Bettelli E, Gao W *et al.* IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. *Nature* 2007; **448**:484–7.
34 Cumberbatch M, Singh M, Bearman RJ *et al.* Impaired Langerhans cell migration in psoriasis. *J Exp Med* 2006; **203**:953–60.
35 Costantino CM, Baecher-Allan CM, Hafler DA. Human regulatory T cells and autoimmunity. *Eur J Immunol* 2008; **38**:921–4.
36 Larsen JM, Bonefeld CM, Poulsen SS *et al.* IL-23 and TH17-mediated inflammation in human allergic contact dermatitis. *J Allergy Clin Immunol* 2009; **123**:486–92.

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CONTACT DERMATITIS

Susceptibility and reactivity in polysensitized individuals following controlled induction

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Background: It is uncertain whether polysensitized patients acquire multiple allergies only because of a high degree of exposure to environmental allergens, or because of being highly susceptible to developing contact allergy.

Objectives: The aim of this study was to investigate and compare susceptibility and reactivity in polysensitized and monosensitized individuals, and in healthy controls.

Patients/methods: We sensitized 66 adult individuals (21 polysensitized, 22 monosensitized, and 23 healthy controls) with diphenylcyclopropenone and assessed challenge responses with visual scoring and ultrasound. We compared sensitization rates using a chi-square test and logistic regression analyses, and calculated linear regression lines of the elicitation responses for each individual. The mean values of the slopes and the intercepts for each group were used to measure the strength of the elicitation response, and were compared using the Mann–Whitney test.

Results: Sensitization ratio was equal in the three groups: 57% for the polysensitized, 59% for the monosensitized, and 65% for the healthy control group. There was a lowered elicitation threshold in the polysensitized group compared with that in the monosensitized and healthy control groups and, although not statistically significant, a stronger elicitation response was observed in the polysensitized group.

Conclusion: Increased reactivity was found in the polysensitized group, demonstrated by a lowered elicitation threshold, compared with that in the monosensitized and healthy control groups.

Key words: contact allergy; elicitation thresholds; polysensitivity; sensitization study.

Conflict of interests: The authors have declared no conflicts.

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Many questions regarding skin sensitization remain unanswered. Why do some people become sensitized to allergens that most people tolerate, and why do some people develop multiple allergies? Polysensitization has been defined as contact allergy to three or more unrelated allergens, as suggested in two recent reviews (1, 2). Prevalence studies have reported polysensitization in 1 in 6 sensitized patients in hospital-based studies (3–5) and 1 in 21 sensitized patient in a general population (6), indicating that polysensitized patients more often have chronic severe eczema requiring hospital attention; moreover, polysensitization is also associated with a long duration of disease (7), but not more generalized dermatitis (8). Polysensitization is encountered more often than would be expected by chance (6, 9). These patients more often develop new positive reactions compared with patients with one or two contact allergies when tested additional times (10). Moreover, polysensitized patients have been shown to produce greater induction and elicitation responses when experimentally exposed to dinitrochlorobenzene (9). Therefore, some regard this group of patients as belonging to a special entity, whereas others regard it a part of a sensitization spectrum resulting from multiple exposures. The objective of this study was to evaluate susceptibility to sensitization, and to evaluate the elicitation

reaction with regard to intensity and variability in thresholds, related to a strong allergen under controlled conditions of exposure.

Material and Methods

Subjects

A total of 66 adult patients were included in the study: 21 polysensitized patients (17 women and 4 men, mean age 51.2 years), 22 monosensitized patients (14 women and 8 men, mean age 48.2 years), and 23 healthy controls (14 women and 9 men, mean age 34.6 years).

Patients were recruited from the Department of Dermato-allergology, Gentofte Hospital, University of Copenhagen, Denmark, and healthy controls via advertisements.

All patients had been patch tested in the department with the European baseline series and, if relevant, additional series dependent on exposure. Patients with positive reactions to three or more allergens on the patch test were allocated to the polyallergic group. Patients with a positive reaction to one allergen on the patch test were allocated to the monoallergic group. Only allergens that were not chemically/structurally related were regarded as new allergies.

Inclusion criterion was 18–65 years of age. Exclusion criteria were as follows: (i) women who were either pregnant or breast-feeding; (ii) use of corticosteroids or other immunosuppressive drugs; (iii) exposure to sunlight 2 weeks before the start of the study; (iv) a history of atopy; (v) occupational contact allergy; (vi) allergy caused by topical treatment; (vii) previous exposure to diphenylcyclopropenone; and (viii) current malignancy or a history of malignancy.

Some of the patients had chronic eczema, but none in an active form and not on the test sites.

The study was approved by the local Ethics Committee, and all subjects gave written informed consent prior to enrollment.

Sensitization and challenge

The sensitization protocol is shown in Fig. 1. All subjects were sensitized with diphenylcyclopropenone in acetone on buttock skin. Petrolatum-backed 11-mm filter discs were soaked in 50 μl of 0.0625% diphenylcyclopropenone 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 discs were left for 48 hr.

Challenges were carried out on the upper inner arm 3 weeks after sensitization, using four concentrations of diphenylcyclopropenone in acetone

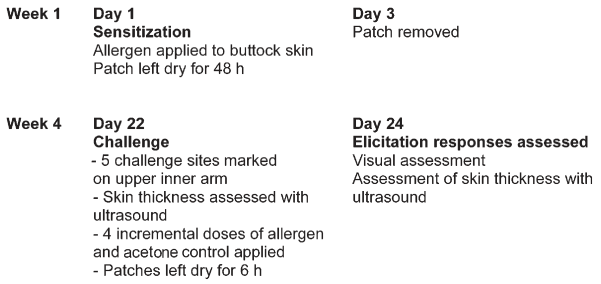

Fig. 1. Diphenylcyclopropenone sensitization protocol.

(0.39, 0.78, 1.56, and 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 hr. The challenge sites were all marked with a surgical skin marker for evaluation 48 hr later.

Visual assessment

The elicitation responses were assessed using a visual score, as suggested by Cooper et al. (11): 1 = no reaction, 2 = mild, macular erythema, 3 = moderate erythema, occasionally with papules, 4 = strong erythematous reaction (including vesicular changes), and 5 = extreme or spreading reaction (including bullous or ulcerative reaction). The final evaluation of the visual clinical assessment was calculated as the sum of scores at the four diphenylcyclopropenone challenge sites of subjects with at least one positive reaction. A visual score of 2 or above was considered a positive reaction.

Ultrasound assessment of skin thickness

Increase in dermal thickness, measured with ultrasound, has been shown to correlate well with the strength of an elicitation reaction (12). In addition to the visual scoring, dermal thickness of each elicitation site was determined using a high-frequency ultrasound scanner (Dermascan, Cortex technology, Horsens, Denmark), and 12-mm scanned images were recorded pre-challenge and post-challenge. Five dermal thickness measurements were taken from each pre-challenge 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. A mean percentage increase in dermal thickness of 10% or more was considered a positive reaction.

Statistical Analysis

Positive elicitation reaction measured with either visual clinical score or ultrasound was categorized a positive sensitization. Group sensitization ratios were

compared using a chi-square test and logistic regression analyses. The outcome of challenge, whether positive or negative, was used as the dependent variable, and allergy status, sex, and age were used as the independent variables. Results were expressed as odds ratios with 95% confidence intervals.

Strength of reactions in the three groups were analysed with both the visual scores and the 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 analysis of the elicitation responses was carried out for each individual, and the mean values of the slopes and the intercepts, used as parameters for strength of the elicitation reaction, for each group were compared using the Mann–Whitney test, as recommended for serial measurements. P-value <0.05 was considered to be statistically significant.

Threshold concentration was defined as the weakest concentration giving a positive response, with either visual clinical score or ultrasound, in a continuous line of positive reactions starting from the highest concentration, and was compared between the groups with the Mann–Whitney test.

All data analyses were performed using SPSS® version 12 (SPSS, Chicago, IL, USA).

Results

Overall sensitization ratio was equal across the three groups: 57% (12/21) for the polysensitized patients, 59% (13/22) for the monosensitized patients, and 65% (15/23) for the healthy control group (Fig. 2).

The percentage increase in dermal thickness correlated well with the dose-dependent clinical scores, based on the visual assessment, and a linear dose-dependent increase in response to diphenylcyclopropenone was observed in all sensitized individuals. Overall, strength of the elicitation responses was stronger in the polysensitized group compared with those in the monosensitized and healthy groups; however, this difference was not evident when analysed statistically. In Fig. 3, the mean visual clinical scores of elicitation reactions at all four diphenylcyclopropenone doses are depicted separately for the three groups. For all four diphenylcyclopropenone doses, the mean strength of the elicitation responses is higher in the polysensitized group compared with that in the other two groups.

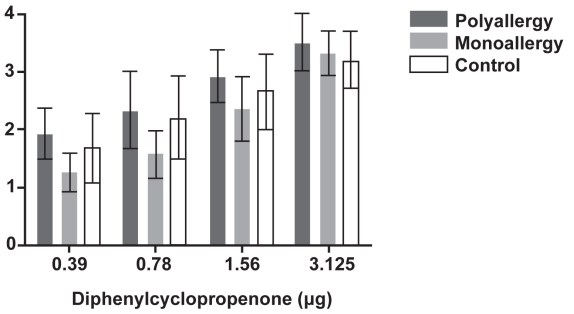

Fig. 3. Response to four incremental challenge doses of diphenylcyclopropenone as assessed by visual clinical scores. Group mean ± standard error of the mean is indicated.

Using the ultrasound data, we compared mean slope and intercept for the groups of the individual regression lines obtained for the elicitation responses to diphenylcyclopropenone. Mean slope and intercept were higher in the polysensitized group compared with that in the monosensitized group: 21.8 versus 18.3 and 45.8 versus 28.1, respectively, but were not statistically different when the Mann–Whitney test was applied, $P = 0.5$ and 0.07, respectively.

Threshold data for the groups are shown in Fig. 4. There was a significantly lower median elicitation threshold dose in the polysensitized group (0.39 µg)

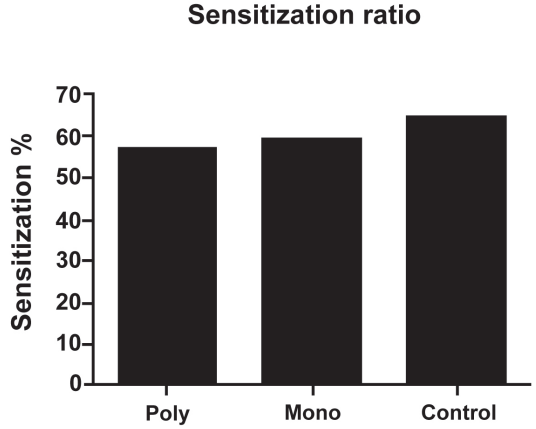

Fig. 2. Sensitization ratio of polysensitized, monosensitized, and healthy control groups.

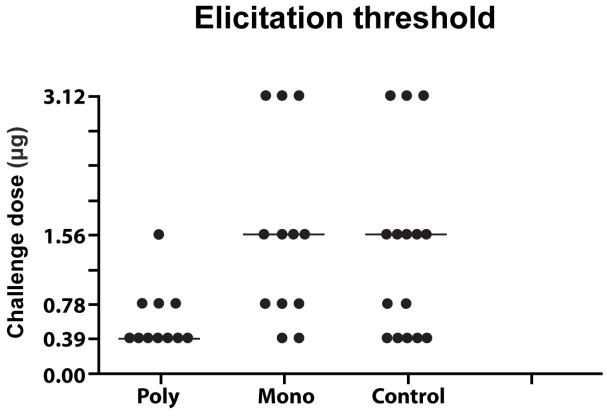

Fig. 4. Elicitation thresholds, defined as the weakest concentration giving a positive response in polysensitized, monosensitized, and healthy control groups. Group median is indicated.

compared with that in the monosensitized (1.56 μg) and healthy control groups (1.56 μg), with $P = 0.005$ and 0.037, respectively. The challenge doses used did not show any irritant response in the unsensitized individuals.

Discussion

In this study, we evaluated the sensitization and elicitation potential in polysensitized individuals, and compared it with that of monosensitized and healthy controls using an experimental sensitization design. We found no difference in the overall sensitization ratio or statistical difference in the strength of elicitation, but we found a lowered elicitation threshold in the polysensitized group compared with that in the monosensitized and healthy control groups.

In 1985, Moss et al. experimentally sensitized polysensitized, nickel allergic and healthy individuals with dinitrochlorobenzene (9). Induction was conducted with three different doses and challenge with four incremental doses of dinitrochlorobenzene. The sensitization ratio increased with the induction dose in all groups. No difference in overall sensitization ratio was found between the groups. Using the strongest induction dose, sensitizing 100% of allergic individuals, Moss et al. furthermore found the response to challenge to be greater in the polysensitized group compared with that in the other two groups. Within the lower induction doses, the challenge data were less clear and interpretation was hampered by the small number of participants.

In accordance with Moss et al., we found no difference in the overall sensitisation ratio between the groups. Sensitizing a relatively large group of individuals with an induction dose sensitizing around 60% of healthy individuals, we found a trend towards greater responses to challenge doses only in the polysensitized group.

Environmental factors are crucial in developing allergy but are not the sole drivers; various studies have pointed to the role of genetic factors in developing contact allergy (13–15). There is no evidence to clarify the relative influence of inherited susceptibility and environmental exposure on the development of contact allergies.

Using an experimental allergen with a sensitization potential higher than most allergens encountered in the environment, we did not find a higher sensitization ratio in a large group of polysensitized individuals compared with that of monosensitized and healthy controls. This finding seemingly contradicts the hypothesis that polysensitized individuals are polysensitized because of an increased susceptibility to developing allergy. However, the relative influence of inherent susceptibility and environmental exposure on developing contact allergy may vary with the induction dose and type of allergen. This is indicated by a study by Walker et al. in which children were sensitized to dinitrochlorobenzene regardless of sensitization status of their parents; however, using the comparatively weaker allergen *N*-nitrosodimethylamine, it was found that children of parents who were sensitized to *N*-nitrosodimethylamine were sensitized significantly more than children of parents who were not sensitized to *N*-nitrosodimethylamine (16). The use of a strong allergen in our study may have hampered our ability to detect differences in overall sensitization potential between the test groups.

Nevertheless, we found a significantly lower threshold to diphenylcyclopropenone challenge in the polysensitized group compared with that of both monosensitized and healthy controls, indicating a greater reactivity in the polysensitized group. In a recent work, using the allergens nickel, methyldibromo glutaronitrile, and *p*-phenylenediamine, no significant difference in elicitation response could be demonstrated between polysensitized and single/double sensitized individuals (17); however, the study carried some inherent limitations in methodological design and test size and the results were regarded only as an exploratory. One limitation that is likely to have influenced the results was the lack of controlled induction. It is well established that the strength of the elicitation response correlates with the induction dose (9, 18). In our study, the induction dose was controlled and equal for all participants; however, the conclusions of our study represent an extrapolation from just one allergen.

A strength in our study is the large and highly selected group of polysensitized individuals. In theory, some polysensitized individuals may not have an increased, inherent susceptibility, but have been exposed to high concentrations of allergens for so long that allergy development was inevitable. To account for this, individuals with occupational allergies and allergies resulting from topical treatment, including leg ulcer patients, were excluded from our study. Furthermore, to account for possible cluster-effects, only allergens that were not chemically/structurally related were regarded as new allergies. None of the participants in the study had a history of atopy.

The reason behind the lower elicitation thresholds in polysensitized patients can only be hypothesized. One explanation could be a shared genetic background, and another could be that the immunological milieu has changed with multiple allergies, whereby an elicitation response is more easily evoked. Whatever the reason, the finding is in accordance with the clinical fact that polysensitized patients have clinical

manifestations in the form of allergic contact dermatitis more often than monosensitized patients and have longer lasting dermatitis (7). This knowledge is important when guiding and informing the polysensitized patients in the clinic.

To conclude, we found no difference in the sensitization ratio, and a stronger trend in the strength of elicitation and a lowered elicitation threshold in polysensitized patients compared with that of monosensitized and healthy controls. Overall, environmental exposure is the most important factor in developing contact allergy. Without environmental exposure, contact allergy cannot be induced; however, seemingly an increased reactivity lowers the threshold for elicitation. The study contributes to the evidence of polysensitization as a phenotype for inherent, increased reactivity.

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References

1. Carlsen B C, Andersen K E, Menné T, Johansen J D. Patients with multiple contact allergies: a review. *Contact Dermatitis* 2008: 58: 1–8.
2. Schnuch A, Brasch J, Uter W. Polysensitization and increased susceptibility in contact allergy: a review. *Allergy* 2008: 63: 156–67.
3. Carlsen B C, Menné T, Johansen J D. Twenty years of standard patch testing in an eczema population with focus on patients with multiple contact allergies. *Contact Dermatitis* 2007: 57: 76–83.
4. Dickel H, Taylor J S, Bickers D R, Merk H F, Bruckner T M. Multiple patch-test reactions: a pilot evaluation of a combination approach to visualize patterns of multiple sensitivity in patch-test databases and a proposal for a multiple sensitivity index. *Am J Contact Dermatitis* 2003: 14: 148–153.
5. Hegewald J, Uter W, Pfahlberg A, Geier J, Schnuch A. A multifactorial analysis of concurrent patch-test reactions to nickel, cobalt, and chromate. *Allergy* 2005: 60: 372–378.
6. Nielsen N H, Menné T. Allergic contact sensitization in an unselected Danish population. The Glostrup Allergy Study, Denmark. *Acta DermVenereol* 1992: 72: 456–460.
7. Carlsen B C, Andersen K E, Menné T, Johansen J D. Characterization of the polysensitized patient: a matched case-control study. *Contact Dermatitis* 2009: 61: 22–30.
8. Carlsen B C, Andersen K E, Menné T, Johansen J D. Sites of dermatitis in a patch test population: hand dermatitis is associated with polysensitization. *Br J Dermatol* 2009: 161: 808–813.
9. Moss C, Friedmann P S, Shuster S, Simpson J M. Susceptibility and amplification of sensitivity in contact dermatitis. *Clin Exp Immunol* 1985: 61: 232–241.
10. Jensen C D, Andersen K E. Course of contact allergy in consecutive eczema patients patch tested with TRUE test panels 1 and 2 at least twice over a 12-year period. *Contact Dermatitis* 2005: 52: 242–246.
11. Cooper K D, Oberhelman L, Hamilton T A, Baadsgaard O, Terhune M et al. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: relationship to dose, $CD1a^{-}DR^{+}$ epidermal macrophage induction, and Langerhans cell depletion. *Proc Nat Acad Sci U S A* 1992: 89: 8497–8501.
12. Kelly D A, Walker S L, McGregor J M, Young A R. A single exposure of solar simulated radiation suppresses contact hypersensitivity responses both locally and systemically in humans: quantitative studies with high-frequency ultrasound. *J Photochem Photobiol* 1998: 44: 130–142.
13. Reich K, Westphal G, König I R, Mössner R, Krüger U, Ziegler A, Neumann C, Schnuch A. Association of allergic contact dermatitis with a promoter polymorphism in the IL16 gene. *J Allergy Clin Immunol* 2003: 112: 1191–1194.
14. Westphal G A, Schnuch A, Moessner R, König I R, Kränke B, Hallier E, Ziegler A, Reich K. Cytokine gene polymorphisms in allergic contact dermatitis. *Contact Dermatitis* 2003: 48: 93–8.
15. Blömeke B, Brans R, Dickel H, Bruckner T, Erdmann S, Heesen M, Merk H F, Coenraads P J. Association between TNFA-308 G/A polymorphism and sensitization to paraphenylenediamine: a casecontrol study. *Allergy* 2009: 64: 279–283.
16. Walker F B, Smith P D, Maibach H I. Genetic factors in human allergic contact dermatitis. *Int Arch Allergy Appl Immunology* 1967: 32: 453–462.
17. Carlsen B C, Fischer L A, Søsted H, Vølund A, Menné T, Johansen J D. Patch test dose-response study: polysensitized individuals do not express lower elicitation thresholds than single/double-sensitized individuals. *Br J Dermatol* 2009: 160: 103–106.
18. Friedmann P S, Moss C, Shuster S, Simpson J M. Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. *Clin Exp Immunol* 1983: 53: 709–715.

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Impaired hapten sensitization in patients with autoimmune disease

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Abstract

An inverse relation between contact allergy and autoimmune diseases is suggested from epidemiological studies. We demonstrate in a controlled experimental sensitization study impaired senisitization to a strong hapten Dipheylcyclopropenone in 23 patients with psoriasis and 22 patients with diabetes compared to 23 healthy controls. All were sensitized on buttock skin and challenged on the upper arm three weeks later. The sensitization ratios were 26%, 36% and 65% for the psoriatic, diabetic and healthy group respectively. Logistic regression analysis gave an odds ratio for a person with psoriasis or diabetes type I of being sensitized to 0.18 (CI 95% 0. 039 - 0.85) P=0.031 and 0.74 (95% CI 0.548 – 1.008) P= 0.056, respectively. Skin biopsies taken from challenged skin were investigated for presentation of FOXP3+ regulatory T cells with immunohistochemistry. A high degree of FOXP3+ cells were found in positive elicitation reactions but only limited numbers in negative elicitation reactions. Skin biopsies from challenged skin were investigated for down regulatory mechanisms with gene expression profiles using microarray technology. No specific mRNA expression was found in the challenged skin of negative elicitation reactions, indicating no sign of active down regulation. The study adds strongly to the evidence of a decreased susceptibility to develop contact allergy in individuals with autoimmune diseases, such as psoriasis.

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 diseases (2-5). Two experimental sensitization studies have shown reduced reactivity to challenge in patients with psoriasis (6, 7), but the ability to become sensitized was not investigated. One study has found reduced sensitization ratio among patients with rheumatoid arthritis (8), but sensitization ratio and reactivity of patients with other autoimmune diseases have never been investigated and the mechanisms behind the apparent impairment in contact allergy remain unknown.

Contact allergy is highly regulated, in part due to regulatory T cells playing a role in diminishing collateral damage and helping in the resolution of ACD. Regulatory T cells may even help in preventing ACD all together, indicated by recent studies showing that in non-allergic individuals antigen-specific regulatory T-cells are activated and found in the challenge sites of non-allergic individuals (9, 10), thus an active down regulation is taking place.

It was the aim of our study firstly 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. Secondly to identify whether or not down-regulatory events was present in the elicitation phase, by investigating skin biopsies taken from elicitation sites with immunohistochemistry and mRNA expression profiles with microarray analysis.

RESULTS

Sensitisation outcome

23 patients with psoriasis, 22 patients with diabetes and 23 healthy controls were sensitized on buttock skin with DPCP and challenged on the upper arm three weeks later. Challenge responses were evaluated with a visual clinical score and ultrasound. A visual score of 2 or above or a mean percentage increase in dermal thickness of 10% or more was considered a positive reaction.

Sensitisation ratios were lower in both the psoriatic and diabetic group compared with the healthy group of subjects. The sensitization ratio was 26% (6/23) 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 gave an odds ratio for a person with psoriasis of being sensitized to 0.18 (CI 95% 0. 039 - 0.85) P=0.031, when adjusted for sex and age. The crude odds ratio for a person with diabetes type I of being sensitized was 0.74 (95% CI 0.548 – 1.008) P= 0.056.

The percentage increase in dermal thickness, 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.

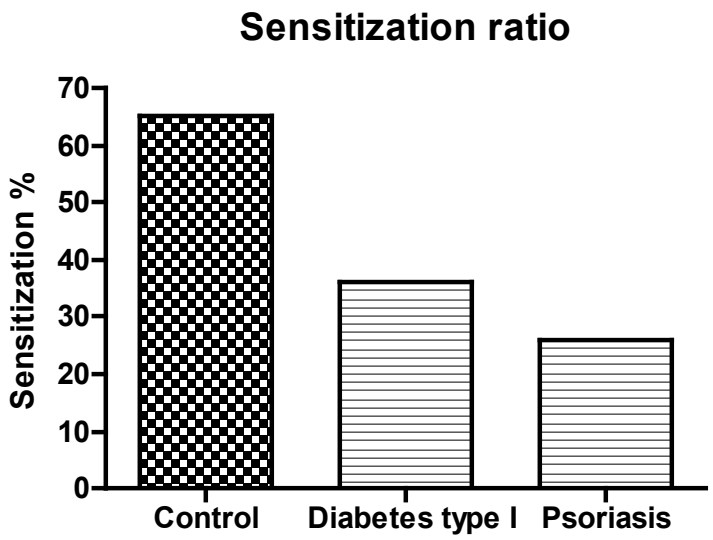


Fig.1. Sensitization ratios of healthy controls (n=23), patients with diabetes type I (n=22) and patients with psoriasis (n=23).

Group	Sens.	Sum Clinical Score Mean +/- s.d	UL regression line mean slope +/- sd	UL regression line mean intercept +/-sd
Healthy	15	9.7 +/- 4.4	23.1 +/- 14.3	59.0 +/- 54.9
Psoriasis	6	8.0 +/- 1.4	22.8 +/- 13.2	30.3 +/-12.6
Diabetes type I	8	9.9 +/- 4.0	15.9 +/- 8.1	51.6 +/- 53.8

Tabl.1 Strength of positive elicitation responses

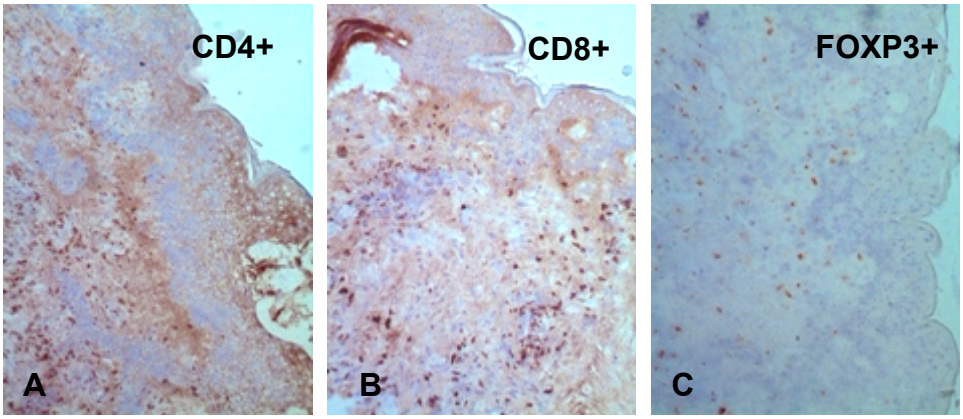
Immunohistochemistry

Biopsies from challenge sites were investigated for cell infiltration including FOXP3+ regulatory T cells. Biopsies were 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. The biopsies were prepared for immunohistochemistry and incubated with anti-CD4, anti-CD8 and anti-FOXP3 antibodies. The degree of infiltration of positively stained cells was scored semi quantitatively using a 5-point scale (grade 0-4).

In all five biopsies from subjects with a positive elicitation reaction, including healthy controls and patients with psoriasis, 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, demonstrated in fig. 4. CD4+ cells and FOXP3+ were mainly distributed in the dermis, with only scattered cells in epidermis. CD8+ cells were also mainly found in 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 a grade 4 infiltration of CD8+ cells, but with very few CD4+ or FOXP3+ cells. The 6 biopsies from subjects with negative elicitation reactions all showed a histological picture of healthy skin; hence there were no sign 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 patients with psoriasis could be made from the infiltration of T cells neither in patients with a positive nor a negative elicitation reaction.

A positive elicitation reaction



A negative elicitation reaction

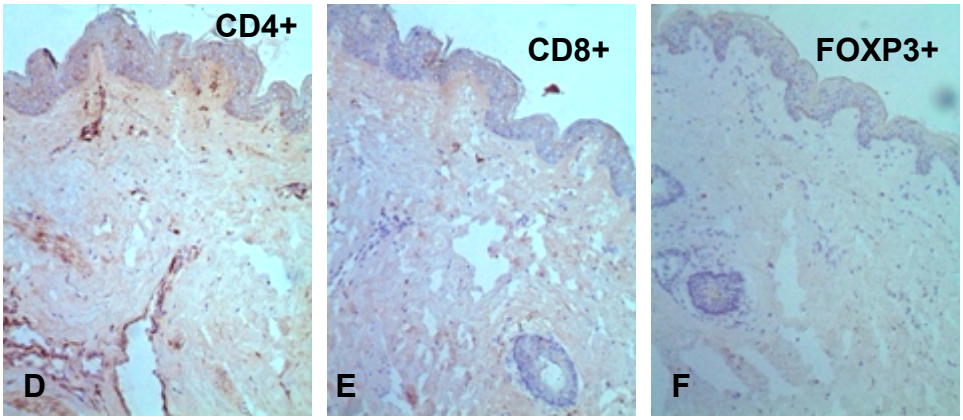


Fig.4. Immunhistochemistry showing a positive elicitation reaction in the same subject A) CD4+ positive cells B) CD8+ positive cells C) FOXP3+ positive cells and immunohistochemistry of a negative elicitation reaction in another subject D) CD4+ positive cells E) CD8+ positive cells F) FOXP3+ positive cells

Gene expression profiles

In order to investigate possible differences in challenge responses between patients with psoriasis and healthy controls, biopsies from both groups with and without positive challenge reactions were selected for gene expression analysis.

Seven patients with psoriasis, two of whom had a positive elicitation reaction and ten healthy controls, five of whom had a positive elicitation reaction were recruited for analysis for gene expression. RNA was extracted from the skin biopsies taken from elicitation sites and a genome wide gene expression analysis carried out using Affymetrix Human Gene 1.0 ST Array. The whole data set and subsets thereof were subjected to Principle Component Analysis (PCA). Fig. 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 dataset respectively. A clear separation of the biopsies along the first dimension is observed. Biopsies from patients with negative clinical elicitation reaction are projected toward 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 clinical positive elicitation reaction from patients with negative elicitation reactions. The group of patients with psoriasis could not be distinguished in the PCA score plot from the 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 overrepresented annotation terms, an annotation analysis was applied. Annotation terms are terms for biological processes defined by the Gene Ontology Consortium. The annotation analysis revealed that terms for biologi-

cal processes related to immune response were overrepresented in the annotation genes defining the negative direction of the first PC axis. Terms related to proliferation were overrepresented in the annotation of genes defining the positive direction of the second PC axis. To further investigate whether or not elicitation reactions were specifically down regulated in patients with psoriasis, probe sets from psoriasis patients as well as healthy individuals with a negative elicitation reaction were selected for further analysis with t-test and subsequent correction for multiple testing with Bonferroni adjustment. When comparing the two groups no significant difference in gene expression was found.

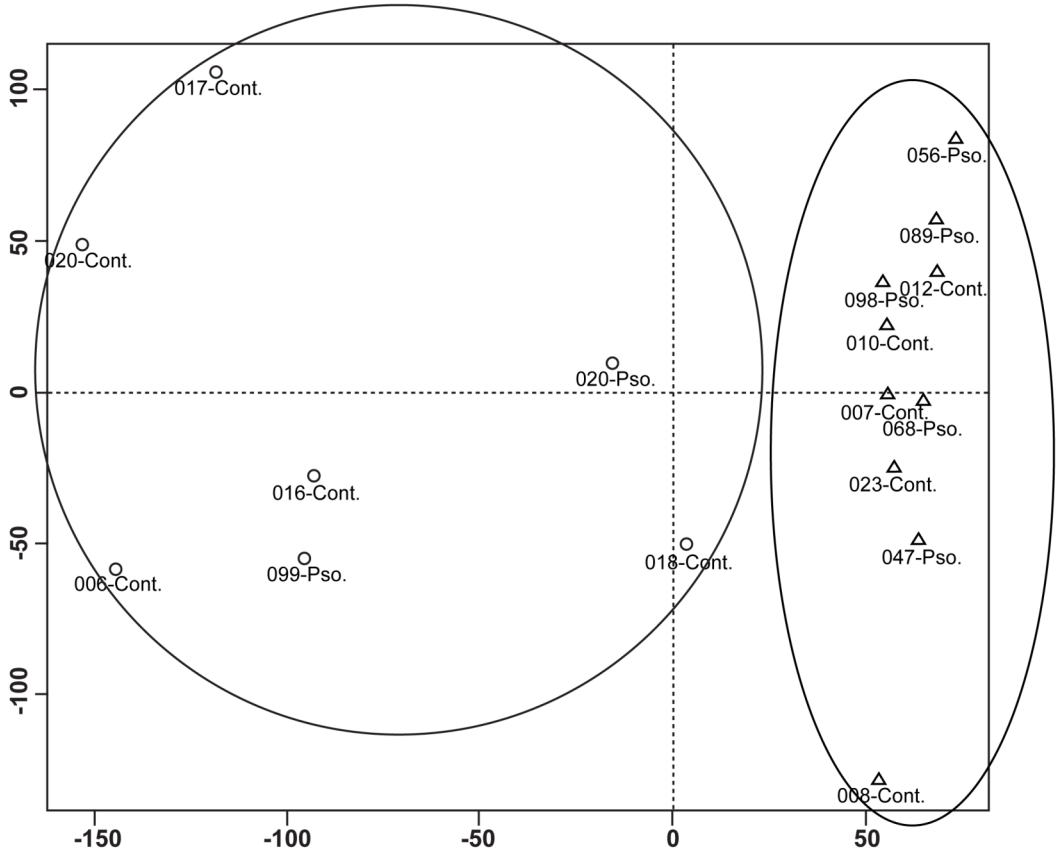


Fig.3. PCA plot, scores for the first and second axes. The projections of samples from subjects with a positive ○ and negative Δ elicitation reactions respectively are indicated by circles.

MATERIAL AND METHODS

Subjects

68 adult patients were included in the study. 23 patients with psoriasis (13 women, 10 men, mean age 50.7), 22 patients with diabetes type I (10 women, 12 men, mean age 40.0) and 23 healthy controls (14 women, 9 men, mean age 34.6).

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 advertisements.

Inclusion criteria were: (i) Age between 18 and 65 years of age. (ii) For patients with psoriasis, a diagnosis of psoriasis clinically verified by a specialist in dermatology and for patients with diabetes, a diagnosis of diabetes type I, insulin dependent. 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 two weeks. (iv) A history of atopy. (v) Previous exposure to DPCP. (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.

Sensitisation

All subjects were sensitised with DPCP in acetone on buttock skin. Petrolatum-backed 11mm filter disks were soaked in 50 µl of 0.0625% DPCP in acetone (25 µg/50 µl). Each filter disc was mounted inside a 12mm aluminium Finn chamber®

and taped to the skin (Scanpor®; Epitest Oy, Tuusula, Finland). The disks were left for 48 hours.

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

Sensitization as well as challenge was done on healthy skin.

Visual assessment

The elicitation responses were assessed using a visual score, as suggested by Cooper and co-workers (11): 1=no reaction, 2=mild, macular erythema, 3=moderate erythema, occasionally with population, 4=strong erythematous reaction (including vesicular changes), 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 with Ultrasound technique has been shown to correlate well with strength of an elicitation reaction (12). In addition to the visual scoring, dermal thickness of each elicitation site was determined using a high-frequency ultrasound scanner (Dermascan, Cortex technology, Horsens, DK).12mm scanned images were recorded pre-challenge and post-challenge. Five dermal thickness measurements were taken from each pre-challenge and post-challenge scan at

fixed distances of 2, 4, 6, 8 and 10mm 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 hours 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 OCT compound (Sakura Finetek, Zoeterwoude, the Netherlands), instantly frozen in liquid nitrogen and stored at –80 ° C until use. Specimens embedded in Tissue Tek and immediately frozen at -80°C in liquid nitrogen, were cut into 5-µm-thick sections, air-dried and fixed in acetone for 5 minutes and kept at -80°C. Endogenous peroxidase activity was blocked by incubation for 5 minutes in peroxidase block, diluted in 0.03% hydrogenperoxid in 95% ethanol. Following rinse with distilled water times three, 0.05 % TBS for 5 minutes and 1% BSA in TBS for 10 minutes, the sections were incubated for 60 minutes at room temperature with the primary antibodies (mouse antihuman) 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-Foxp3 antibody (clone 236 A/E7;1: 50), obtained from eBioscience. After rinsing with TBS, a secondary antibody (EnVision+ kit K4004, DAKO) labeled with horseradish peroxidase was applied for 30 minutes at room temperature. Enzymatic activity was revealed by a 5–10 minute incubation with 3, 3'-diaminobenzidine (DAB) + substrate-chromogen (EnVision+ kit K4007, DAKO), which results in a brown-colored precipitate at the antigen site. Counterstaining was performed with aqueous Mayer's hematoxylin (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 ten 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 immediately placed 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, Netherlands) and homogenized with a rotor stator (Polytron PT_{3000} Kinamatica AG, Buch & Holm A/S, Denmark). Total RNA was then extracted using the *mir*Vana^{TM} Isolation Kit (Applied Biosystems) following the manufacturer's specifications. RNA concentration was determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilming-

ton, 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, Calif) containing probe sets of approximately 26,000 genes. Generation of cDNA, biotin-labeled cRNA and GeneChip hybridization was performed by the RH Microarry Centre at Rigshospitalet (Copenhagen, Denmark). Samples were labeled according to manufactures guidelines using GeneChip Whole Transcript Sense Target Labeling Assay (Affymetrix, Santa Clara, CA, USA). In short 100 nano grams of total RNA was transcribed into cDNA using T7-$(N)_6$ primers. Antisense RNA is generated by in vitro transcription of the cDNA, which are used as template to generate dUTP-containing sense cDNA fragments. Following the removal of RNA fragments, the sense cDNA fragments were terminal labeled with biotin. The labeled samples were hybridized to the Human Gene 1.0 gene ST array (Affymetrix, Santa Clara, CA, USA). The arrays were washed and stained with phycoerytrin 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 Gene Chip® protocol.

Data Analysis

Clinical data

Group sensitisation rates were compared by using a chi-square test and logistic regression analyses. The outcome of challenge, 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).

Strength of reactions in the three groups were 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. With the ultrasound data linear regression analysis 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, for each group were compared using a Mann-Whitney test, as recommended for serial measurements. P-value $\leq 0{\cdot}05$ was considered to be statistically significant.

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

Immunohistochemistry data

The degree of infiltration of positively stained cells was scored semi quantitatively using a 5-point scale 0 = none, 1=few positive, 2 =some positive, 3=many positive, 4=highly positive.

To detect significant differences between mean values of the groups the Mann–Whitney U-test was applied. Significance was determined with a p value of less than

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

Microarray data

A single $\log_2$ scale expression measure for each probe set was attained from the low-level data files (CEL files), using the robust multiarray analysis procedure with quantile normalization (Irizarry reference) 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 overrepresented GO terms using Fishers exact test for proportions with Bonferroni correction for multiple test. Mapping of probe Ids to Go terms was performed with the annaffy package from Bioconductor (13).

To further analyze the possible difference in gene expression between patients with psoriasis and healthy controls, probe sets from psoriasis patients as well as healthy individuals with a negative elicitation reaction were selected for further analysis with t-test and subsequent correction for multiple testing with Bonferroni adjustment.

Discussion

In a controlled experimental sensitization study using the strong allergen DPCP, with a sensitization potential stronger than most allergens encountered in the environment, we are the first to show significantly lower sensitization ratios in two large groups of patients with psoriasis and diabetes type I respectively, compared with

healthy controls. The result is supporting recent epidemiological studies showing an inverse relation between contact allergy and several autoimmune diseases; psoriasis, diabetes type I, inflammatory bowel disease and rheumatoid arthritis (2-5).

In 1965 Epstein and Maibach sensitized 13 patients with psoriasis and 32 healthy controls with the strong allergen Dinitrochlorobenzene (DNCB) and found a slightly reduced sensitization ratio in the psoriatic group, but interpretation was hampered by the small study sample (14). Two other experimental studies sensitizing psoriatic patients with DNCB have been conducted. Both studies used a high allergen dose for sensitization, sensitizing nearly all participants and hence they focused on the degree of challenge responses only. Moss et al. found reduced challenge reactions compared to the healthy controls (6) and Obalek and co-workers reported a higher threshold in patients with psoriasis compared to healthy controls (7). 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 likely that the effect is dependent upon the sensitization dose, which in our study was deliberately chosen relatively low sensitizing only 65% of the healthy group in order to study differences in sensitization potentials.

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

One study has demonstrated a reduced sensitization ratio in patients with Rheumatoid arthritis using DNCB (8) indicating that the impaired reactivity to hapten could be common for autoimmune diseases. The autoimmune diseases psoriasis, diabetes type I, rheumatoid arthritis and inflammatory bowel diseases have been linked

through common clinical traits, genetic polymorphisms and immunological pathways (15-17). 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, though a complex process involving integrated signals from the innate and adaptive immune system, in which T cells during the induction phase 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; 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 (18-20). 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 (9,10), 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. With immunohistochemical staining we found no difference in cell infiltration between healthy skin and skin from challenge sites of non-responders after 48 hours, indicating that the lack of response was not due to an active down-regulation. In agreement with this, we did not find any significantly up or down regulation of gene expression in the challenge sites of non-responders after 48 hours. Furthermore, mRNA expression in elicitation responses of patients with psoriasis and healthy controls could not be distinguished, neither 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 dysfunctional in suppressing auto-antigen specific effector T cells in various autoimmune diseases (21-24), but their regulation of environmental antigens were not investigated in these studies.

The significance of a Th17 profile in autoimmune diseases is well established through multiple areas of research, reviewed by Steinmann (25) and patients with autoimmune diseases have been demonstrated to have higher than normal levels of circulating Th17 cells and cytokines such as IL-17, IL-6, IL-21, IL-22 and IL-23 (26). We hypothesize that the highly Th17 directed cytokine milieu in patients with autoimmune diseases is interfering with the mounting of a contact allergic response. This could be due to interference in the differentiation of naïve T cells to become effector or memory T cells necessary for the contact allergic reaction or due to regulation of antigen presenting cells. Interestingly Brandt et al. demonstrated in a murine study, that short-time incubation of in vitro generated DC with IL-21 significantly reduced their potential to induce an antigen-specific CD8+ T cell proliferation (27, 28). Antigen presenting cells, especially Langerhans cells (LC), play a pivotal role in the sensitization phase of contact allergy, as they are responsible for the processing, transport, and presentation of allergens to naïve T lymphocytes in the skin draining lymph nodes. Cumberbatch et al. found that the function of epidermal Langerhans cells and specifically LC mobilization and migration, is profoundly impaired in the uninvolved skin of psoriasis patients compared with the skin of healthy volunteers (29). The authors hypothesised that this could be due to disease progression characterized by systemic changes that affect LC function. The systemic changes could very well be due to a Th17 skewed milieu found not only in patients with psoriasis but patients with other autoimmune diseases as well.

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

References

1. Thyssen JP, Linneberg A, Menné T et al. The epidemiology of contact allergy in the general population--prevalence and main findings. *Contact Dermatitis*. 2007; 57:287-99
2. Bangsgaard N, Engkilde K, Thyssen JP, Linneberg A, Nielsen NH, Menné T, Skov L, Johansen JD. Inverse relationship between contact allergy and psoriasis: results from a patient- and a population-based study. Br J Dermatol. 2009 Nov; 161(5):1119-23.
3. Engkilde, K, Menné, T. and Johansen, J. D. PB.39 Inverse association between rheumatoid arthritis and allergic contact dermatitis. Contact Dermatitis 58 (Suppl. 1.), 68-69. 27-5-2008. Ref Type: Abstract
4. Engkilde K, Menné T, Johansen JD. Inflammatory bowel disease in relation to contact allergy: a patient-based study. *Scand J Gastroenterology*. 2007; 42: 572-6.
5. Engkilde K, Menné T, Johansen JD. Inverse relationship between allergic contact dermatitis and type 1 diabetes mellitus: a retrospective clinic-based study**.** *Diabetologica* 2006; 49: 644-7.
6. Moss C, Friedmann PS, Shuster S. Impaired contact hypersensitivity in untreated psoriasis and the effects of photochemotherapy and dithranol/UV-B. *Br J. Dermatol.* 1981; 105: 503-8.
7. Obalek S, Haftek M, Slinski W. Immunological studies in psoriasis. The quantitative evaluation of cell-mediated immunity in patients with psoriasis by experimental sensitization to 2, 4-dinitrochlorobenzene. *Dermatologica*. 1977; 155:13-25
8. Epstein WL, Jessar RA.Contacttype delayed hypersensitivity in patients with rheumatoid arthritis. Arthritis Rheum 1959 Apr;2(2):178-81
9. Cavani A, Nasorri F, Ottaviani C, Sebastiani S, De Pità O, Girolomoni G. Human CD25+ Regulatory T Cells Maintain Immune Tolerance to Nickel in Healthy, Nonallergic Individuals. J Immunol. 2003 Dec 1; 171(11):5760-8.
10. Moed H, von Blomberg BM, Bruynzeel DP, Scheper RJ, Gibbs S, Rustemeyer T. Regulation of nickel-induced T-cell responsiveness by CD4+CD25+ cells in contact allergic patients and healthy individuals. Contact Dermatitis. 2005 Aug; 53(2):71-4.

11. Cooper K.D, Oberhelman L, Hamilton T.A, Baadsgaard O, Terhune M et al. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: Relationship to dose, $CD1a^{-}$ DR^{+} epidermal macrophage induction, and Langerhans cell depletion. Proc.Natl.Acad.Sci 1992; 89; 8497-8501
12. Kelly DA, Walker SL, McGregor JM, Young AR. A single exposure of solar simulated radiation suppresses contact hypersensitivity responses both locally and systemically in humans: quantitative studies with high-frequency ultrasound. Journal of photochemistry and Photobiology. 1998; 44: 130-142
13. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004;5(10):R80.
14. Epstein WL, Maibach HI. Immunological competence of patients with psoriasis receiving cytotoxic drug therapy. Arch Dermatol. 1965 Jun; 91:599-606.
15. Henseler T, Christophers E. Disease concomitance in psoriasis. *J Am Acad Dermatol*. 1995; 32:982-6
16. Wolf N, Quaranta M, Prescott NJ, Allen M, Smith R, Burden AD, Worthington J, Griffiths CEM, Mathew CG, Barker JN, Capon F,Trembath RC. Psoriasis is associated with pleiotropic susceptibility loci identified in type II diabetes and Crohn disease J Med Genet 2008; 45:114–116.
17. Langrish CL, Chen, Blumenschein WM. Et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005; 201:233-40
18. Kish DD, Gorbachev AV, Fairchild RL.CD8+ T cells produce IL-2, which is required for CD (4+) CD25+ T cell regulation of effector CD8+ T cell development for contact hypersensitivity responses. J Leukoc Biol. 2005 Sep; 78(3):725-35
19. Ring, S., S. J. Oliver, B. N. Cronstein, A. H. Enk, and K. Mahnke. 2009. CD4+ CD25+ regulatory T cells suppress contact hypersensitivity reactions through a CD39, adenosine-dependent mechanism. J. Allergy Clin. Immunol. 123:1287-1296.
20. Ring S et al. CD4+CD25+ regulatory T cells supress contact hypersensitivity reactions by blocking influx of effector T cells into inflamed tissue. Eur J Immunology 2006; 36:2981-2992
21. Sugiyama H, Gyulai R, Toichi E, Garaczi E, Shimada S, Stevens SR, McCormick TS, Cooper KD. Dysfunctional blood and target tissue CD4+CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. J Immunol. 2005 Jan 1; 174(1):164-73.
22. Viglietta, V., C. Baecher-Allan, H. L. Weiner, and D. A. Hafler. 2004. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med. 199:971*.

23. Kriegel, M. A., T. Lohmann, C. Gabler, N. Blank, J. R. Kalden, and H. M. Lorenz. 2004. Defective suppressor function of human CD4+ CD25+regulatory T cells in autoimmune polyglandular syndrome type II. *J. Exp. Med.199:1285*.
24. J. M. Lawson, J. Tremble, C. Dayan,H. Beyan, R. D. G. Leslie, M. Peakman and T. I. M. Tree. Increased resistance to CD4+CD25hi regulatory T cell-mediated suppression in patients with type 1 diabetes.Clin Exp. Immunology. 2008
25. Steinman L. A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell–mediated tissue damage. *Nature Med*. 2007; 13:139-45. Kagami S, Rizzo HL, Lee JJ, Koguchi Y, Blauvelt A.
26. Circulating Th17, Th22, and Th1 cells are increased in psoriasis.J Invest Dermatol. 2010 May; 130(5):1373-83. Epub 2009 Dec 24.
27. Brandt K, Bulfone-Paus S, Jenckel A, Foster DC, Paus R, Rückert R. Interleukin-21 inhibits dendritic cell-mediated T cell activation and induction of contact hypersensitivity in vivo.J Invest Dermatol. 2003 Dec; 121(6):1379-82.
28. Brandt K, Bulfone-Paus S, Foster DC, Rückert R. Interleukin-21 inhibits dendritic cell activation and maturation. Blood. 2003 Dec 1;102(12):4090-8
29. Cumberbatch M, Singh M, Dearman RJ, Young HS, Kimber I, Griffiths CE. Impaired Langerhans cell migration in psoriasis. J Exp Med. 2006 Apr 17; 203(4):953-60.