

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 diphenylcyclopropanone 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. © John Wiley & Sons A/S, 2010.

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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 μ l of 0.0625% diphenylcyclopropenone in acetone (25 μ g/50 μ l). 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

Week 1	Day 1 Sensitization Allergen applied to buttock skin Patch left dry for 48 h	Day 3 Patch removed
Week 4	Day 22 Challenge - 5 challenge sites marked on upper inner arm - Skin thickness assessed with ultrasound - 4 incremental doses of allergen and acetone control applied - Patches left dry for 6 h	Day 24 Elicitation responses assessed Visual assessment Assessment of skin thickness with ultrasound

Fig. 1. Diphenylcyclopropenone sensitization protocol.

(0.39, 0.78, 1.56, and 3.125 μ g/15 μ l) 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 diphenylcyclopropanone was observed in all sensitized individuals. Overall, strength of the elicitation responses was

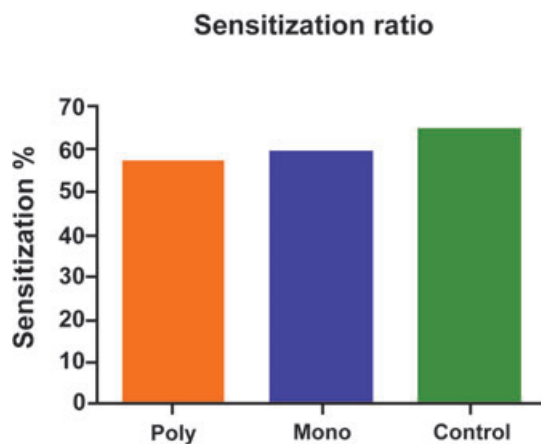


Fig. 2. Sensitization ratio of polysensitized, monosensitized, and healthy control groups.

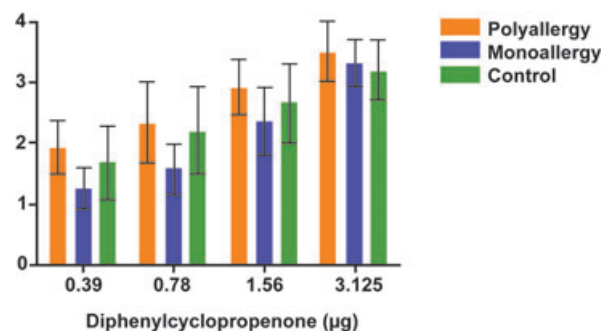


Fig. 3. Response to four incremental challenge doses of diphenylcyclopropanone as assessed by visual clinical scores. Group mean $\pm$ standard error of the mean is indicated.

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 diphenylcyclopropanone doses are depicted separately for the three groups. For all four diphenylcyclopropanone doses, the mean strength of the elicitation responses is higher in the polysensitized group compared with that in the other two groups.

Using the ultrasound data, we compared mean slope and intercept for the groups of the individual regression lines obtained for the elicitation responses to diphenylcyclopropanone. 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)

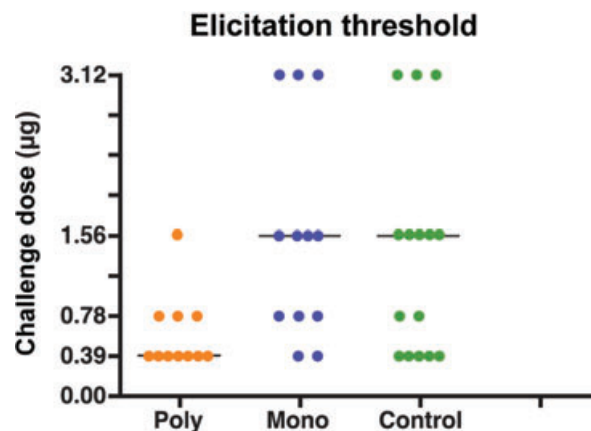


Fig. 4. Elicitation thresholds, defined as the weakest concentration giving a positive response in polysensitized, monosensitized, and healthy control groups. Group median is indicated.

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 diphenylcyclopropanone 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, methyl dibromoglutaronitrile, 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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