

Elicitation of contact hypersensitivity after repeated suberythematous exposures of humans to solar simulated radiation: number of epidermal Langerhans cells

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Ultraviolet radiation suppresses contact hypersensitivity (CHS). The role of epidermal Langerhans cells (LCs, CD1a⁺) in the elicitation phase of CHS is uncertain. To assess the effect of low-doses of solar simulated radiation (SSR) on LC numbers at the CHS elicitation site, 3 groups (each about 30 volunteers) were whole-body irradiated with suberythematous SSR on 2, 10 or 30 consecutive days before sensitization with diphenylcyclopropanone. Another group was not irradiated. Elicitation of CHS took place 3 weeks later with subsequent evaluation by visual scoring and spongiosis grade. CD1a⁺ cells in the epidermis from the elicitation site were counted. No difference in CHS intensity between the unirradiated controls and all 3 irradiated groups was found, but a significant negative correlation between the spongiosis grade and the number of SSR exposures was shown. The number of epidermal CD1a⁺ cells in the 10- and 30-day groups was reduced compared with the unirradiated group, and the 30-day group had significantly fewer than the 10-day group. Low daily doses of SSR induce suppression of CHS, leading to depletion of LCs at the CHS elicitation site. The effect on the CHS and LCs is cumulative, indicating that photoadaptation for these parameters does not develop over the 30 day irradiation period.

Key words: contact hypersensitivity; dendritic cells; immunosuppression; langerhans cells; solar simulated radiation. © Blackwell Munksgaard, 2007.

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The Langerhans cells (LCs) are bone-marrow derived dendritic cells (DCs), and they represent the major antigen presenting cells of the epidermis. They are the only cells in the epidermis to express CD1a, apart from the related indeterminate cells that account for less than 2% of the total CD1a⁺ cell population in normal skin (1). LCs are regarded as sentinels of the adaptive immune system, responding to changes in the microenvironment, and interacting with external antigens. In response to contact sensitizers, many LCs are induced to migrate from the skin and arrive in the draining lymph nodes via the afferent lymph (2–5). During this journey, they become mature immunocompetent DCs that are able to present the hapten effectively to responsive T lymphocytes. Their critical role in the induction (afferent) phase of contact hypersensitivity (CHS) has been

clearly shown (6–10). In contrast, their function is not established for the elicitation (efferent) phase of CHS when the same sensitizer is re-applied to the skin and a close interaction between the infiltrating T cells and antigen presenting cells is presumably required. While some reports indicate that LCs are major players in determining the extent of the elicitation response (11–14), others find the opposite (15).

It is known that exposure to ultraviolet radiation (UVR) prior to sensitization with a hapten induces suppression of CHS. Many of the details of this complex process have been determined (16, 17). However, in many instances such studies have been performed on animal models or in human subjects using a single erythematogenous dose of UVR, containing a high proportion of ultraviolet light B (UVB) (18–20). In real life most individuals

go outside and are exposed to sunlight, containing at most 6% UVB, for short periods of time on a daily basis in the summer months. They respond by tanning and epidermal thickening. We have tried to mimic this type of natural exposure more closely by repeatedly irradiating healthy volunteers with a suberythral dose of solar simulated radiation (SSR), containing about 4% UVB, on a daily basis for up to 30 days. The dose of SSR chosen was small, representing less than a 0.3 minimal erythema dose for subjects of phototype II and III; this would be achieved after approximately 15 min on a clear day around midday in summer in mid-Europe. We have shown that such exposure results in suppression of CHS, based on either the visual score at the lowest challenge dose of the sensitizer, or on histological features in the epidermis at a higher challenge dose (21). Furthermore, the effect was cumulative so that the number of days of exposure correlated with the degree of down-regulation of the CHS response. Now we extend this study by assessing the influence of repeated suberythral doses of SSR, administered before sensitization of individuals with the contact allergen, diphenylcyclopropanone (DPCP), on the number, and distribution of epidermal CD1a⁺ LCs during the elicitation phase of CHS.

Materials and Methods

Subjects and SSR

The study group consisted of 138 healthy volunteers with either second or third skin phototype, as assessed by the Fitzpatrick scoring system (22). These phototypes were selected as they are found in the majority of the population in Central Europe. The subjects were recruited following advertising in the local newspapers and within the University, and were without any skin or other disease and not receiving any medication. Individuals exposed to high-doses of sunlight or sunlamps within 2 months prior to the study or previously sensitized with DPCP were excluded. To decrease the influence of natural solar radiation, all the procedures were performed between November and February. Each volunteer gave written informed consent before entry into the study and underwent a thorough physical examination and blood count. The experimental plan was approved by the local Ethics Committee.

The volunteers were divided into groups as shown in Table 1. Group A consisted of 38 unirradiated subjects, Group B of 28 subjects irradiated on 2 consecutive days, Group C of 30 subjects irradiated on 10 consecutive days and Group D of 32 subjects irradiated on 30 consecu-

Table 1. Clinical characteristics of the study groups

Group	Number of subjects	Mean age years $\pm$ SD	Sex F/M	Phototype II/III
A: unirradiated, sensitized	38	24.9 $\pm$ 3.1	16/22	18/20
B: 2 $\times$ SSR, sensitized	28	26.3 $\pm$ 6.9	15/13	13/15
C: 10 $\times$ SSR, sensitized	30	25.0 $\pm$ 5.8	13/17	21/11
D: 30 $\times$ SSR, sensitized	32	26.8 $\pm$ 7.4	15/17	22/11
E: unirradiated, non-sensitized	10	26.7 $\pm$ 4.8	4/6	4/6

F, female; M, male; SSR, solar simulated radiation.

tive days. The irradiations were whole-body in 2 half-wall cabinets, excluding the genital area, with 1.2 standard erythema dose (SED), where 1 SED is equivalent to an erythema radiant exposure of 100 J/m² of SSR on each occasion. The SSR was generated by 100 W Cleo Natural lamps (Philips, Eindhoven, the Netherlands) giving an even field of irradiance (4% UVB, 96% UVA) of about 4.95 mW/cm² on the skin surface at 20 cm from the source. The emission spectrum of the Cleo Natural lamp is shown in Fig. 1. Measurement of the intensity of the lamps was performed using a Solar Light 3 D ultraviolet meter (Solar Light Co., Philadelphia, USA). A further group (Group E) consisted of 10 individuals who were neither irradiated nor sensitized, and who acted as controls for the histological and immunohistochemical studies.

Sensitization and elicitation of CHS

The method described in (21) was followed. In outline, DPCP (Fluka Chemie GmbH, Buchs, Switzerland) was dissolved in acetone just before use. The subjects were sensitized on the left irradiated (Groups B–D) or non-irradiated (Group A) buttock skin using two 7 mm petrolatum-backed filter discs, 1 soaked in 20 μ l 0.1% DPCP (20 μ g), and the other in 20 μ l acetone (to test for irritation

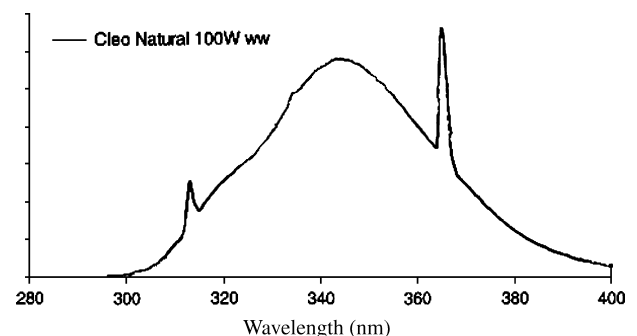


Fig. 1. Emission spectrum for the Cleo Natural 100 W lamp.

because of the solvent). The irradiated subjects were sensitized 1 D after the final exposure to SSR. The filters were mounted inside 8 mm aluminium Finn chambers (Epitest Ltd Oy, Tuusula, Finland), which were taped to the skin with hypoallergenic Scanpore tape and left in place for 2 D. The volunteers were requested to keep the patch dry for 2 D, after which the patch was removed. 3 weeks later, all the volunteers in Groups A–D received an antigenic challenge on the unirradiated upper inner left arm skin using a disc soaked in 20 µl acetone containing 3.2 µg DPCP. 1 additional patch contained acetone to differentiate CHS from irritation. The patches were put on the skin in Finn chambers, held in place with tape, and were removed 6 hr later. The CHS response was evaluated after 2 D by a subjective visual scoring system where 0=no reaction, 1=macular erythema, 2=erythema with infiltration, 3=erythema with infiltration and papules or vesicles, and 4=bullous reaction. The visual score for all subjects was determined by one dermatologist who did not know to which group the individual belonged at the time of the examination.

Histological examination of CHS and CD1a staining

A 3 mm-punch skin biopsy was taken from the elicitation site in each subject 2 D after challenge. Additionally, biopsies were taken from the same skin site of the non-sensitized, unirradiated volunteers in Group E. The tissue specimens were fixed in 10% buffered formaldehyde, routinely processed for paraffin embedding and 3–4 µm sections cut. These were dewaxed and stained with haematoxylin and eosin. 10 serial sections were evaluated for spongiosis from each biopsy by 2 independent pathologists at a magnification of $\times 400$ (Olympus Bxsystem microscope, Olympus Optical Co., LTD, Tokyo, Japan). Histological morphometry was performed using an image analysis system consisting of an IBM-compatible computer equipped with an optical mouse, Indeo Fast card (frame grabber, true-colour, real-time), produced by Indeco (Taiwan), and colour TV camera (Panasonic, Japan) linked to a Carl Zeiss Jeneval microscope (Germany). This system was programmed (programme MultiScan 8.08, Computer Scanning System, Poland) to calculate the intensity of spongiosis graded as 0-none, 1-slight oedema with no intraepidermal vesicles, 2-oedema with single intraepidermal vesicles, 3-severe oedema, and multiple intraepidermal vesicles.

In addition, immunohistochemical staining for CD1a was performed on sections of the biopsies. Briefly, sections were dewaxed, rehydrated, and

rinsed in distilled water. Endogenous peroxidase was blocked by 3% H₂O₂. After rinsing in buffer, the sections were incubated for 30 min at room temperature with mouse monoclonal anti-human CD1a antibody, dilution 1 : 200 (Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK). After 3 rinses in buffer, the sections were incubated with the detection kit (anti-mouse EnVision+System-HRP, DakoCytomation, Glostrup, Denmark) and the colour was developed with 3,3'-diaminobenzidine (DakoCytomation). The sections were then counterstained with hematoxylin, dehydrated and mounted in Canadian balsam. Negative controls consisted of the same process but omitted the primary antibody. The number of cells expressing CD1a (membrane pattern of staining) in the epidermis was counted by a computer-image programme (sis Analysis, Olympus, Japan). At least 2 sections from each subject were examined at $\times 400$ magnification, and the mean number of positive cells per mm² calculated.

Statistical analysis

Statistical analysis of the data was performed using the Mann–Whitney test and non-parametric correlation of Spearman. A *P* value of less than 0.05 was considered significant.

Results

Contact hypersensitivity

The CHS was first assessed visually using the scoring system outlined in the Materials and Methods and no statistical differences were found between Group A (unirradiated, sensitized) and Groups B–D (irradiated, sensitized) (results not shown). When the CHS score was simplified by treating it as a binomial trait where 0 represents no reaction and 1 represents any response, a negative trend between the score and the number of days of irradiation was noted but this was insignificant (*P* > 0.05 for all comparisons, Spearman non-parametric test). These results are shown in Table 2.

Table 2. CHS response and occurrence of spongiosis (simplified scoring system in both cases) to diphenylcyclopropanone challenge in sensitized individuals following repeated SSR

Group	Percentage with any CHS response	Percentage with any feature of spongiosis
A, unirradiated	89.5	85.0
B, 2 $\times$ SSR	85.6	77.3
C, 10 $\times$ SSR	83.4	68.8
D, 30 $\times$ SSR	81.3	50.0

CHS, contact hypersensitivity; SSR, solar simulated radiation.

CHS was also evaluated by assessing the degree of spongiosis in skin biopsies collected from the elicitation site. The highest grade of 3 was found in 37.5% of individuals in Group A (unirradiated) and in only 10.5% of individuals in Group D ($30 \times$ SSR). When the spongiosis grade was simplified by treating it as a binomial trait where 0 represents no reaction and 1 represents any feature of spongiosis, a response was observed in 85% of subjects in Group A and 50% in Group D (Table 2). If the number of days of SSR and the spongiosis score were considered as continuous variables, a significant negative correlation between these 2 factors was shown ($R = -0.52$, $P < 0.05$, Spearman non-parametric test). No spongiosis was evident in the biopsies from the subjects in Group E (unirradiated, non-sensitized).

CD1a⁺ cells

In the epidermis of the Group E subjects (unirradiated, non-sensitized), CD1a⁺ cells were

observed mainly in the suprabasal layer, while in the Group A subjects (unirradiated, sensitized), following elicitation, they were scattered throughout the epidermis and around intraepidermal vesicles. After exposure to SSR and elicitation, the CD1a⁺ cells migrated to lower parts of the epidermis and were mainly seen in the suprabasal layer in Group D. Furthermore, the positive cells tended to be less dendritic than in the unirradiated epidermis. Representative sections from Groups A–E are shown in Fig. 2.

The daily SSR induced a gradual reduction in the number of CD1a⁺ cells in the epidermis at elicitation (Fig. 3). This decrease was significant when Group A was compared with Groups C and D. In addition, the number in Group D was significantly lower than in Group C and was no different from the number in Group E.

The mean number of CD1a⁺ cells for subjects within a Group who developed a CHS response was then compared with the mean number in subjects in the Group who did not develop a response,

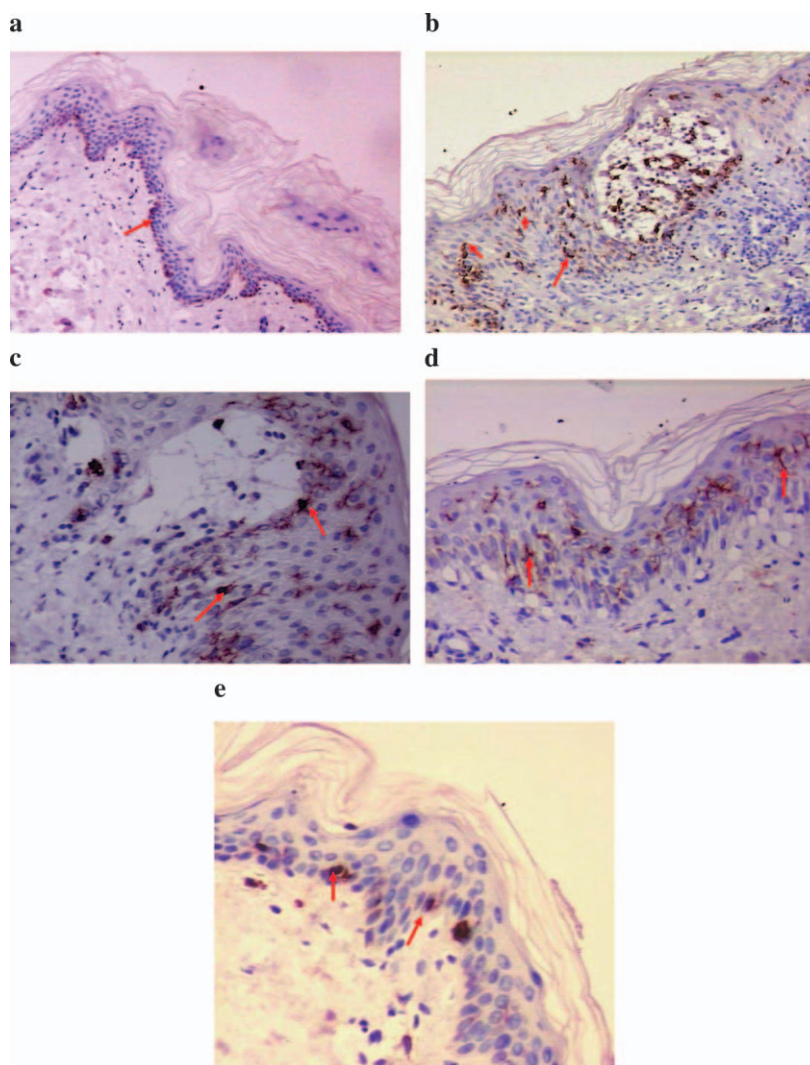
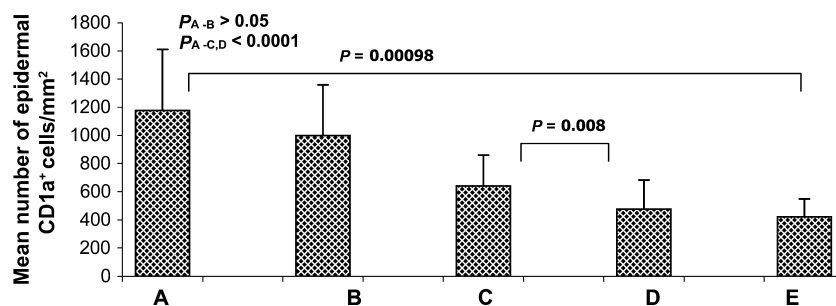


Fig. 2. Immunohistochemical staining showing CD1a⁺ cells (arrows) in representative skin biopsies during the elicitation phase of contact hypersensitivity from **a)** Group E (unirradiated, non-sensitized; $\times 200$), **b)** Group A (unirradiated, sensitized; $\times 200$), **c)** Group B ($2 \times$ solar simulated radiation (SSR), sensitized; $\times 400$), **d)** Group C ($10 \times$ SSR, sensitized; $\times 400$), and **e)** Group D ($30 \times$ SSR, sensitized; $\times 600$).

Fig. 3. Number of CD1a⁺ epidermal cells during the elicitation phase of contact hypersensitivity in Groups A (unirradiated, sensitized), B (2 × solar simulated radiation (SSR), sensitized), C (10 × SSR, sensitized), D (30 × SSR, sensitized), and E (unirradiated, non-sensitized). The mean ± SD for each group is shown.



as judged by the visual score. In each case there was always a higher number in the individuals who developed CHS than in those who did not, but these differences were not significant (results not shown). Even after pooling the results from all the Groups, no significant difference was found in the CD1a⁺ cell number between subjects with a CHS response and those who had no response (839 cells/mm² versus 742 cells/mm², $P > 0.05$).

When the spongiosis score was treated as a binomial trait, where 0 represents no spongiosis and 1 represents any reaction, significant differences were seen between the mean CD1a⁺ cell number in individuals within each Group with no reaction and the ones with the features of spongiosis. In detail: Group A 576 cells/mm² (0 spongiosis) versus 1280 cells/mm² (1 spongiosis), Group B 585 cells/mm² (0 spongiosis) versus 1119 cells/mm² (1 spongiosis), Group C 411 cells/mm² (0 spongiosis) versus 724 cells/mm² (1 spongiosis), and Group D 361 cells/mm² (0 spongiosis) versus 591 cells/mm² (1 spongiosis). Thus, the number was always higher in subjects where spongiosis developed ($P < 0.01$ for all comparisons).

Discussion

Previously we used the same irradiation protocol, prior to sensitization with DPCP, to show suppression of the CHS response (21). The down-regulation was indicated by the visual score at the lowest challenge dose of DPCP (0.4 µg) only, but was particularly evident when skin biopsies, collected from the elicitation site, were evaluated for features of spongiosis. Using this method of assessment, the intensity of spongiosis was lowered by the SSR at all the challenge doses of DPCP (0.4–3.2 µg). We confirm this finding here as, although the SSR did not affect the visual CHS score significantly at the challenge dose of 3.2 µg DPCP, it did result in less spongiosis. In addition, there was a significant negative correlation between the number of days of SSR and the spongiosis score. Thus, small repeated exposures to SSR led to increasing suppression of the CHS response as measured by spongiosis, and photoadaptation for this parameter did not develop.

The elicitation phase of CHS is thought to require close contact between antigen presenting cells and infiltrating T cells. The antigen presenting cells could reside in the epidermis or dermis or migrate from the blood. Whether LCs act as major players in this process is uncertain (12, 14, 15). Our results indicate that LCs are recruited into the epidermis as a consequence of CHS elicitation as the numbers were threefold higher in the sensitized individuals (Group A) compared with the non-sensitized individuals (Group E).

The involvement of the CD1a⁺ cells in the development and resolution of the elicitation phase of CHS has been shown in 1 detailed phenotypic study (13). In addition, the participation of dermal DCs of myeloid origin and of plasmacytoid DCs in antigen presentation during elicitation was indicated in the same report (13), but it was concluded that mature LCs are the most probable cell type to present hapten to the antigen-specific T cells and thus, are the ones that are responsible for initiating the elicitation of CHS. Tsuruta et al. (9) have also shown that LCs are activated in the epidermis during CHS elicitation.

In the present study, we found that the number of LCs in the epidermis during the elicitation phase of CHS steadily decreased as the number of daily exposures to SSR increased. Therefore, SSR reduced LC numbers after challenge with DPCP, and no photoadaptation regarding this effect was apparent. It should be noted that the dose of SSR selected was small and 1 that could be experienced by many subjects living in mid-latitudes under natural conditions. The mechanism involved in regulating LC numbers during elicitation following prior exposure to SSR is unknown but must be systemic as the elicitation took place on an unirradiated body site.

Finally, our results show that the reduction in the CD1a⁺ numbers correlated with the reduction in the spongiosis score in the irradiated Groups. Furthermore, in all the groups, there were fewer CD1a⁺ cells in the subjects in whom no spongiosis was induced by challenge compared with those in whom spongiosis occurred. These findings provide some evidence that the epidermal CD1a⁺

cells play a key role at the elicitation stage in determining the extent of the CHS response.

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