

General discussion



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The aim of our study was to unravel the pathogenesis of PLE by investigating the supposition that PLE is caused by an impairment in UV-B-induced immunosuppression. This hypothesis runs as a continuous thread through this thesis. The results obtained show that a defect in certain UV-induced cellular responses in the skin, i.e. an impaired Langerhans cell migration and a lack of influx of neutrophils into the epidermis of the uninvolved skin of PLE patients. Our data provide evidence for a disturbance of the relationship between erythema and immunosuppression in PLE. In this chapter the data described in the previous sections will be integrated and discussed in an overall synthesis of a model of the early pathogenic steps in PLE.

EFFECT OF UV-B RADIATION ON CD11b+ CELLS

Cooper and co-workers (Hammerberg *et al*, 1996b; Kang *et al*, 1994; Meunier *et al*, 1995) concluded from their experiments that interleukin (IL)-10 producing CD11b+ macrophage-like cells were involved in UV-B-induced immunosuppression. Treatment with anti-CD11b antibodies resulted in a reversion of this immunosuppression (Hammerberg *et al*, 1996a). Therefore we hypothesized that these cells were absent or non-functional in PLE patients.

Initially we tested several UV exposure regimens on healthy individuals to reproduce the data found by Cooper and co-workers. Their observation of a nearly complete depletion of Langerhans cells and an influx of CD11b+ cells into the epidermis after exposure of the skin to 4 MED (Minimal Erythema Dose) UV-B, was used as a guideline. At first, dorsal skin of healthy volunteers was exposed to 4 MED (Philips TL12 lamps). However, the skin area and the UV dose gave variable results on Langerhans cell depletion and influx of CD11b+ cells. A UV dose of 6 MED on buttock skin instead of dorsal skin gave more consistent results, comparable to that reported by the other investigators (**chapter 2**). Cooper and co-workers defined the MED as the UV dose causing a clear erythema with defined borders, while we defined the MED as the UV dose causing a just perceptible redness of the skin. Barr *et al* (1999) calculated that a dose of 4 MED according to Cooper and co-workers was comparable to 5.6 MED, according to our definition.

Consecutively, buttock skin of PLE patients (with normal and extreme sunburn-sensitivity) and healthy individuals was exposed to 6 MED. CD11b+ cells were observed in the unexposed and UV-exposed skin of healthy individuals as well as PLE patients. The number of CD11b+ cells was even larger in PLE patients with an extreme sunburn sensitivity (low MED). However, hardly any CD11b+ cells infiltrated the UV-exposed epidermis of PLE patients compared to healthy controls. Judging by the large number of CD11b+ cells in the

dermis of PLE patients, the hypothesis that a mere lack of these cells would cause the suspected failure in immunosuppression in these patients proved wrong. However, the lack of these cells migrating into the epidermis was highly significant. The CD11b+ population in the skin of PLE patients consisted predominantly of macrophages (CD68+) (**chapter 2**). In healthy individuals the dermal and epidermal CD11b+ cells in the UV-exposed skin were primarily neutrophils (elastase+) (in agreement with Strickland *et al* (1997) and Teunissen *et al* (2002)).

The question remained whether the macrophages in the UV-exposed skin of PLE patients were functional in producing the immunosuppressive cytokine IL-10. A small number of dermal neutrophils and CD36+ macrophages in the skin of healthy individuals and PLE patients expressed the immunosuppressive cytokine IL-10 in their cytoplasm (as detected by immunohistochemistry on microscopic sections). In contrast, infiltrating neutrophils in the epidermis of healthy controls expressed IL-10. Although the CD11b+ population in PLE patients is distinct from healthy controls, the difference in IL-10 expression is restricted to infiltrating (epidermal) neutrophils. This suggests that a lack of IL-10-expressing epidermal neutrophils is an aberrant UV response that contributes to the pathogenesis of PLE (**chapter 5**).

UV-B-INDUCED DEPLETION OF LANGERHANS CELLS

An immunohistochemical staining for Langerhans cells (CD1a) was performed on skin biopsies from (un)exposed buttock skin to check the reproducibility of our irradiation protocol and to compare it with the protocol used by Cooper and co-workers. Unexpectedly, we noticed a striking difference in the UV-induced depletion of Langerhans cells between PLE patients and healthy controls. Whereas the Langerhans cell depletion was nearly complete after 6 MED UV-B in healthy individuals, a considerable number of epidermal Langerhans cells was still present in the skin of PLE patients. Initially, we referred to these cells as 'UV-resistant'. However, when PLE patients were exposed to an even larger UV dose (8.4 MED) a greater number of Langerhans cells disappeared from the epidermis. This indicated that these cells were not UV-resistant but relatively less sensitive to UV-B radiation with regard to their migratory response (**chapter 2 and 5**).

The MED of PLE patients with a normal MED was in the lower 50 percentile of the MED range of healthy controls. Therefore one might assume that the impaired Langerhans cell depletion in PLE patients is mainly caused by an on average lower UV dose. However, no correlation could be found between the decrease in Langerhans cell number and the absolute UV exposure. Furthermore, a PLE patient receiving the same absolute UV dose as a healthy control still had a considerably smaller decrease in the number of epidermal Langerhans cells.

The next step was to establish the mechanism underlying the impaired Langerhans cell depletion. To this end we first determined what mechanism was involved in UV-induced Langerhans cell disappearance in healthy individuals. The best studied and most likely mechanisms involved in this depletion were apoptosis and migration. Apoptosis was determined by using the TUNEL assay (identifying late apoptosis by labeling of DNA strand breaks) and by staining of active-caspase 3, a protease involved in the apoptotic cascade. A substantial number of apoptotic cells was detected in the UV-exposed skin of healthy volunteers, however no apoptotic Langerhans cells could be found. Migration of Langerhans cells was confirmed using a newly developed method to trap migrating Langerhans cells in the fluid of a blister raised on the UV-exposed skin (**chapter 3**). Migrating Langerhans cells could also be detected in the blister fluid of the UV-exposed skin of PLE patients, although the number of cells was significantly smaller than in healthy controls (**chapter 4**). PLE patients do have apoptotic cells in their UV-exposed skin but again no apoptotic Langerhans cells could be observed (data not shown). The number of apoptotic cells, predominantly keratinocytes (sunburn cells), in the UV-exposed skin of PLE patients is variable but less than in healthy controls. It seems, though, that the number of apoptotic cells is related to the UV-dose. However, this has to be confirmed in a follow-up study. In conclusion, the impaired UV-B-induced Langerhans cell depletion in PLE patients is completely attributable to an impairment in migration.

Cytokines such as IL-1- α and IL-1- β , tumour necrosis factor (TNF)- α and also IL-18 have been described to be involved in Langerhans cell migration (Cumberbatch *et al*, 1997a; Cumberbatch *et al*, 1997b; Cumberbatch *et al*, 2001). For that reason we investigated the expression of these cytokines in (un)exposed skin of PLE patients and healthy controls (**chapter 5**). The number of cells expressing IL-1- β and TNF- α was less in the UV-exposed skin of PLE patients compared to healthy controls. In contrast to healthy individuals, PLE patients had no TNF- α -expressing neutrophils in their UV-exposed epidermis. The impaired Langerhans cell migration in PLE patients appears, therefore, to be related to a diminished expression of the migration-inducing cytokines IL-1- β and TNF- α .

LANGERHANS CELL MIGRATION AND IMMUNOSUPPRESSION

How can a deficiency in Langerhans cell migration be linked to an impairment in immunosuppression? Although UV-induced Langerhans cell migration has been found to be associated with immunosuppression, it had not been proven that this constitutes a causal relationship.

In experiments with mice deficient in nucleotide excision repair (NER) we demonstrated that a defect in transcription-coupled repair (TCR) resulted in an enhanced local, but not systemic immunosuppression as well as in an enhanced UV-induced Langerhans cell depletion (**chapter 6**). These observations are indicative of a relationship between UV-B-induced

Langerhans cell depletion and local immunosuppression. Hence, our finding of an impairment in UV-induced Langerhans cell migration in PLE patients would suggest an impaired local immunosuppression, as confirmed by a recent study (van de Pas *et al*, *in press (Journal of Investigative Dermatology)*).

MED AND IMMUNOSUPPRESSION

A relationship between MED and immunosuppression was confirmed in humans by Kelly *et al* (2000), who showed that a sensitivity to sunburn is associated with a susceptibility to UV-induced local suppression of contact hypersensitivity (CHS). A lower MED correlated with an increased susceptibility to UV-induced immunosuppression. This correlation between erythema and immunosuppression appears to be disturbed in PLE patients. As mentioned before, the MED of most PLE patients is in the lower range of the MED of healthy individuals. Furthermore, the immune responses in the UV-exposed skin of PLE patients e.g. immunosuppression, Langerhans cells migration and the expression of migration-inducing as well as Th2-skewing cytokines (epidermal IL-4 and IL-10 expression), are reduced compared to healthy individuals. In addition, irradiation of the skin of PLE patients with 8.4 MED instead of 6 MED increased Langerhans cell depletion and the expression of IL-1- β , TNF- α and IL-4 (**chapter 5**). Taken together, these observations suggest that the erythema response in PLE is too strong in comparison to the immunosuppressive response. Thus, the relationship between UV-B-induced erythema and immunosuppression is disturbed in PLE. Another indication for a disturbed relationship is given by the observation that supplementation of dietary fish oil or phototherapy given to PLE patients to prevent a pathological skin reaction to UV exposure, results in a decreased UV-erythema sensitivity (increase of the MED) (Boonstra *et al*, 2000; Rhodes *et al*, 1994; Rhodes *et al*, 1995). Apparently, an elevation of the threshold for the erythema response normalizes the balance with respect to the immunosuppressive response.

DNA DAMAGE AND LANGERHANS CELL MIGRATION

We demonstrated that only a minority of migrating Langerhans cells in the blister fluid of the UV-B-exposed skin had UV-specific DNA damage (**chapter 3 and 4**). The predominance of Langerhans cells without detectable DNA damage could not be explained by an artifact of the suction blister technique or by an influx of new dendritic cells from the bloodstream or hair follicles, because these cells were not found in blister fluid of the unirradiated skin. Moreover, we observed an enrichment of UV-damaged Langerhans cells in the blister roofs of the UV-B exposed skin. Hence, it appeared that undamaged or DNA-repaired Langerhans cells exhibited a selective responsiveness to migratoin signals. Repair of UV-B-induced DNA damage, e.g. cyclobutane pyrimidine dimers (CPDs), could then be a prerequisite for Langerhans cell migration. To investigate the relationship between DNA repair and Langerhans cell migration more closely we exposed transgenic mice with defects in nucleotide excision repair (NER) to different doses of UV-B radiation and studied the epidermal depletion of NLDC145+ Langerhans cells (**chapter 6**). CSB mice deficient in transcription-coupled repair (TCR) and XPA mice deficient in TCR and global genome repair (GGR) showed an enhanced UV-induced depletion of Langerhans cells compared to GGR-deficient XPC mice and the respective wildtype (wt) littermates. Correspondingly, the CSB and the XPA mice proved to be much more sensitive to the induction of local immunosuppression by UV exposure than the XPC and wt mice. The sunburn sensitivity of XPA and CSB is also much higher than XPC and wt mice. At very low UV doses, where XPC and wt show no detectable reaction at all, the CSB and XPA mice already show Langerhans cell migration and local immunosuppression. This suggests that the persistence of a low level of damage in active genes triggers migratory signals (presumably from keratinocytes) to which the Langerhans cells react. The low level of DNA damage in the Langerhans cells does apparently not obstruct their migration. The fact that Langerhans cells react even at higher UV doses in XPC mice, indicates that a persistence of higher levels of DNA damage in non-transcribed strands does not hinder migration either. This indicates that, at least in mice, DNA repair does not seem to be a prerequisite for UV-induced Langerhans cell depletion. However, a persistence of a high level of DNA damage in transcribed strands could perhaps hinder the migration of Langerhans cells, but a suitable model to test this in transgenic mice is not available.

LANGERHANS CELL ACTIVATION AND MATURATION

Langerhans cells persisting in the epidermis of PLE patients after UV-B exposure might present antigen there instead of in the lymph nodes. This antigen presentation at an aberrant location might contribute to the pathogenesis of PLE. In order to present antigen efficiently Langerhans cells need to upregulate their expression of costimulatory molecules as well as

the number of antigen-HLA-DR complexes on their membrane. Furthermore, responding cells, e.g. T-cells, need to be present to carry out a specific immune response. No epidermal Langerhans cells in the UV-exposed skin of PLE patients expressed costimulatory molecules (**chapter 4**). However, the number of epidermal HLA-DR+ Langerhans cell was increased after UV exposure. Additionally, hardly any CD3+ T-cells could be detected in the epidermis of the UV-exposed skin of PLE patients. Taken together, these data demonstrate that antigen presentation in the epidermis of the UV-B-exposed skin of PLE patients most likely does not occur.

Langerhans cells expressing costimulatory molecules and HLA-DR as well as T-cells could be observed in the dermis of the UV-exposed skin of PLE patients and healthy individuals. The absolute number of activated and matured (CD83+) dermal Langerhans cells was substantially larger in PLE patients compared to healthy controls. Illicit antigen presentation could therefore occur in the dermis, with a greater likelihood in PLE patients.

SKEWING TOWARDS A TH1 OR TH2 RESPONSE

Cytokines are important in determining the outcome of an immune response, e.g. a type 1 (Th1) or a type 2 (Th2) response, and most likely as a result, influence the induction of PLE. In healthy individuals UV-B radiation provokes the development of Th2 cells while suppressing Th1 cells (Simon *et al*, 1990; Simon *et al*, 1991). A first immunohistochemical exploration of the cytokine profiles in the skin of PLE patients revealed that the Th2-skewing cytokine IL-4 was expressed in fewer cells in the UV-exposed epidermis of PLE patients when compared to healthy controls (**chapter 5**). The number of cells expressing Th1-skewing cytokines IL-12, interferon (IFN)- γ and IL-6 did not differ between PLE patients and healthy individuals. Overall, the cytokine profile in the UV-exposed skin of PLE patients appeared to be shifted more towards Th1 in comparison to healthy individuals.

FUNCTIONAL TESTING

An impairment in immunosuppression can be tested functionally by immunization or challenge with a contact allergen on an irradiated site (local immunosuppression) or on an unirradiated site after irradiation of another skin area (systemic immunosuppression). The severity of the contact hypersensitivity reaction (CHS) in terms of erythema, edema and the appearance of papules or vesicles can be used as a measure for immune reactivity. Antigens, not yet encountered by the immune system (causing immunization), as well as recall antigens (causing a challenge reaction) can be used as contact allergens. Application of immunizing contact allergens like DNCB or DCP not only encountered ethical objections but also carried the risk of sometimes provoking severe, traumatic skin reactions. Therefore,

we used different concentrations (0.5% and 2%) of the recall antigen nickelsulphate (NiSO_4) in petrolatum, which is also used in the clinic (at a concentration of 5%) for assessment of CHS. Before testing (nickel allergic) PLE patients we first wanted to establish a consistent standardized reaction in healthy individuals who had a positive skin reaction to nickel. In accordance with the UV exposure protocol in this project we exposed buttock skin of seven healthy individuals to 6 MED UV-B (Philips TL12 lamps) and applied different concentrations of nickelsulphate to the UV-B-exposed and unexposed skin 48 hours post irradiation. Following the classical CHS protocol the allergen remained on the skin for 48h and the reaction was measured 24h after removal of the allergen. Assessment of CHS was done by eye and with a chromameter. We did not observe a consistent reaction in healthy individuals ($n = 7$). CHS reactions of the UV-exposed skin fluctuated from an immunosuppressive reaction to an induction of immune reactivity compared to the unexposed skin.

The increased skin reaction to NiSO_4 after UV exposure was not anticipated as UV radiation is known to suppress delayed type (type IV) hypersensitivity reactions like these (Damian *et al*, 1999). However, not only a type IV hypersensitivity reaction has been described for nickel but also a type I IgE-mediated hypersensitivity reaction (Estlander *et al*, 1993). An additional assessment of the skin reaction at 2h after application of the contact allergen might therefore have given a more reliable definition of the reaction in some individuals. Another parameter that had not been taken into account was the menstrual cycle of the participants (all were female). The menstrual cycle might be of influence on skin reactivity (Agner *et al*, 1991; Agner, 1992). Damian *et al* (1999) were able to suppress a CHS on nickelsulphate with UV radiation. However, they used a different UV exposure regimen and applied the antigen immediately after UV exposure.

The inconsistent and sometimes unpleasant reactions made us decide to discontinue these experiments. In the meantime van de Pas *et al* (*in press* (*Journal of Investigative Dermatology*)) were performing experiments in the United Kingdom on the suppression of immunization against DNCB. PLE patients and healthy volunteers were exposed to solar-simulating radiation prior to application of DNCB onto the buttock skin. They showed that UV-induced local immunosuppression was impaired in PLE patients compared to healthy individuals after exposure to 0.6 MED and 1 MED, but not after exposure to 2 MED.

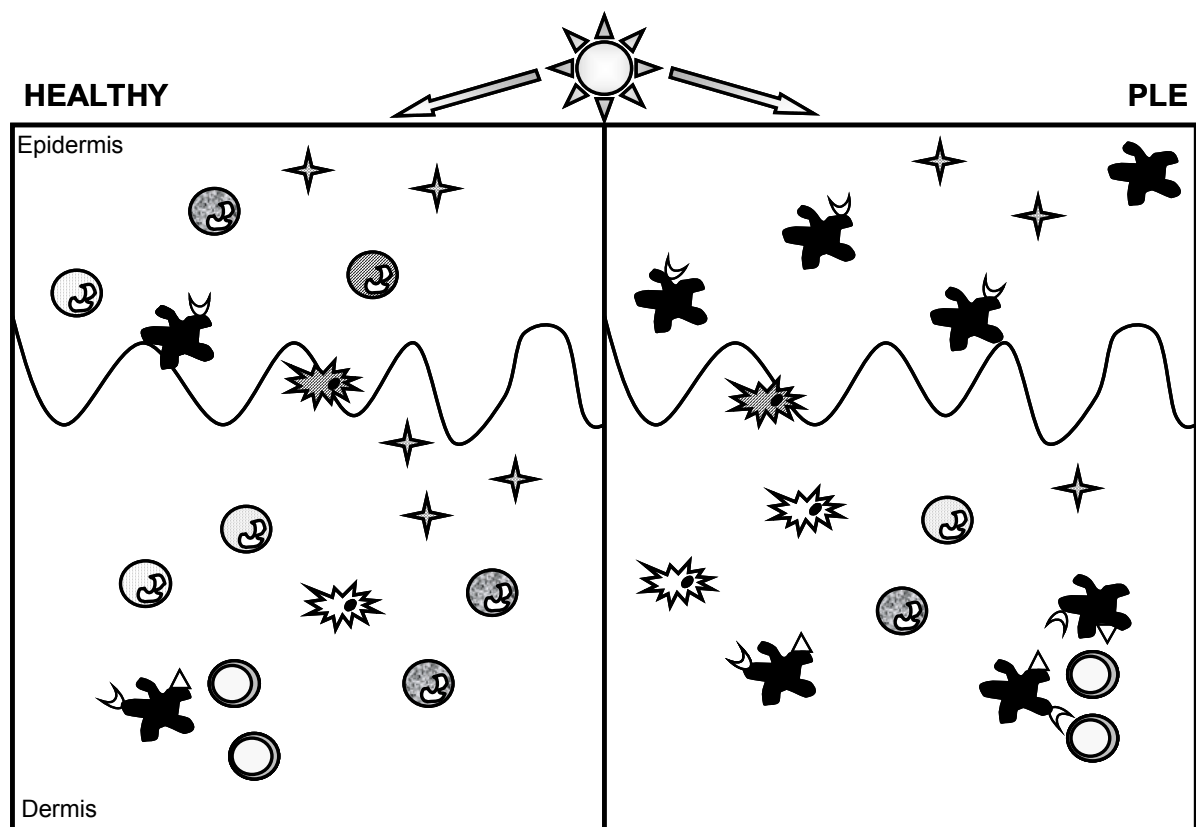
These results imply that there is a dose range in PLE patients over which the immunosuppression is lacking in comparison to the inflammatory (erythematous) response. Although these experiments have not measured the allergic response to UV radiation itself, one could envisage that there is a corresponding "UV dose-window" over which the PLE patients can develop an allergic reaction to UV exposure. It could then be speculated that the allergic response would not develop if the UV exposure were high enough, resulting in a severe sunburn instead.

Although the efflux of Langerhans cells and influx of neutrophils we observed at 6 MED in the PLE patients were low in comparison to the healthy volunteers, they may have been strong

enough to attain to an adequate immunosuppression, as measured by van de Pas *et al* (*in press (Journal of Investigative Dermatology)*).

EARLY EVENTS IN THE PATHOGENESIS OF PLE

A model of the pathogenesis of PLE based on the aberrant responses in the skin to UV-B radiation, as described in this thesis, is presented in Figure 1. In this figure the effect of UV-B radiation on the skin of PLE patients is therefore compared to that in healthy controls.



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| ★ = Langerhans cell | ⊕ = IL-4+ neutrophil | ★ = CD11b+ CD68+ macrophage |
| △ = costimulatory molecules | ⊕ = TNF-α+ neutrophil | ★ = IL-10+ CD36+ macrophage |
| ☾ = HLA-DR | ⊕ = IL-10+ neutrophil | ○ = T-cell |
| ★ = IL-1-β + cell | | |

Figure 1, Model of the early steps in the pathogenesis of PLE. After UV exposure [☀] Langerhans cells [★] still persist in the skin of PLE patients (right panel) in contrast to healthy individuals (left panel). Fewer dermal cells expressing IL-1-β [✦] together with a decreased number of TNF-α-expressing neutrophils [⊕] in the dermis and an absence of TNF-α-expressing cells in the epidermis (above the curved line) lie at the root of this impaired Langerhans cell migration in PLE patients. This deficient Langerhans cell migration correlates with an impairment in UV-induced local immunosuppression in PLE patients. Langerhans cells in the epidermis of PLE patients do not express the activation markers CD86, CD40, CD54 or the maturation marker CD83 but these cells do express HLA-DR [☺] after UV exposure. Dermal Langerhans cells express costimulatory molecules [△] as well as HLA-DR and are therefore capable of presenting (neo-)antigens to dermal T-cells [⊙], thus activating these T-cells. Activated dermal Langerhans cells are present in larger numbers in PLE patients compared to healthy individuals. A decreased number of IL-4-expressing neutrophils [⊕] (without a difference in the expression of Th1-skewing and pro-inflammatory cytokines) skews the balance to a type 1 T-cell response in PLE patients. The CD11b+ cell population in PLE patients consists predominantly of macrophages [☄], while in healthy individuals this population is dominated by neutrophils. The immunosuppressive cytokine IL-10 is expressed by infiltrating neutrophils [⊕] and by a minority of the macrophages [☄]. The difference in IL-10 expression between PLE patients and healthy individuals is restricted to infiltrating neutrophils. Taken together, it appears that the normal, healthy immunosuppressive response to UV radiation is reduced in PLE patients while the erythematous response is increased (possibly by an increased expression of prostaglandin E₂). This shift in immune responses together with the possibility of an activation of Th1 cells (instead of Th2 cells) in the dermis by slowly migrating Langerhans cells may underlie the pathogenesis of PLE.

PLE DEMARCATION FROM OTHER PHOTODERMATOSES

In our first study on the pathogenesis of PLE we included PLE patients with a normal MED as well as a low MED. Later on we focussed on PLE patients with a normal MED because there are no healthy individuals with a low MED who can be included as proper controls for PLE patients with a low MED. At first, we defined a pathological skin reaction in PLE patients clinically as papules, vesicles and eczema on sun-exposed areas of the skin. It appeared that an eczematous reaction to UV radiation in the PLE patients we included, was restricted to PLE patients with a low MED. Furthermore, a skin reaction to UV radiation in patients with photosensitive eczema at an early stage often resembles a PLE reaction with papules and vesicles which later develops into an eczematous lesion. To circumvent possible confounding from eczematous reactions we restricted the definition of PLE to papules and vesicles, excluding any history of (non-)photosensitive eczema. All PLE patients with a normal MED that were included fitted this definition.

These issues on MED and eczematous reactions highlight controversies on the definition of PLE. PLE may well be a diagnosis that entails different forms of photodermatoses. Some structure might be created by a division according to susceptibility to sunburn, sensitivity to different wavelengths, type of reaction (papules and vesicles versus eczematous), extent of the disease (seasonally dependent or not) and maybe even gender. Thus, one could construct the diagram in figure 2 to delineate the relationships between various forms of photodermatoses. The proposed demarcations and relationships between the separate photodermatoses are still very much open to debate, and need to be substantiated by well targeted future research.

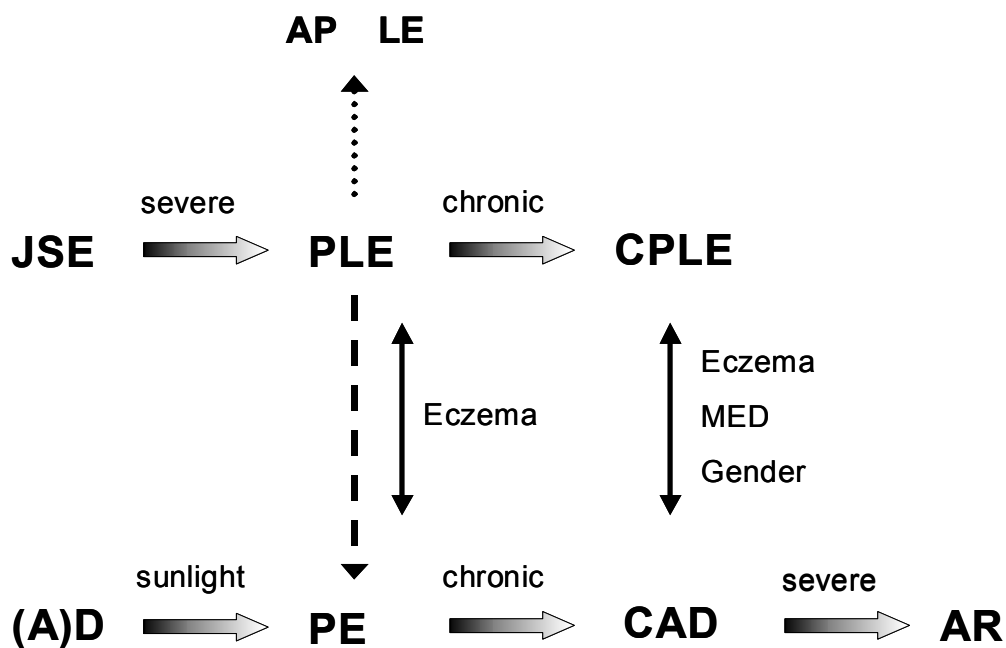


Figure 2, A hypothetical diagnostic diagram (constructed by Soe Janssens, Frank de Gruijl and Wendy Kölgen). This diagram consists of two main branches. The first branch represents a sunlight-induced pathological skin reaction dominated by papules and vesicles. The course of the condition in this branch could start with juvenile spring eruption (JSE). In this diagnosis lesions are commonly restricted to the sun-exposed ears. If severity increases this disorder passes in adulthood into a PLE with a seasonal dependent (predominantly spring and summer) appearance of lesions on sun-exposed areas of the body. Skin disorders like actinic prurigo (AP) and lupus erythematoses (LE) need to be excluded from this diagnosis, although PLE has been reported to precede LE in some patients. When seasonal dependence is lost and skin lesions persist, this can be diagnosed as chronic PLE (CPLE). The other branch in the diagram is dominated by the appearance of eczematous lesions. A photo-provoked eczema could arise de novo or (atopic) dermatitis ((A)D) may evolve into a photosensitive eczema (PE) when the eczema is aggravated by sunlight. PE lesions often first appear as papules and vesicles and might therefore initially be (mis)diagnosed as PLE (). If --> these photosensitive eczematous lesions persist a chronic actinic dermatitis (CAD) develops. An actinic reticuloid (AR), characterized by three diagnostic criteria (see introduction), will develop when CAD

increases in severity. The major difference between PLE and PE () is most likely the clinical appearance of lesions: PLE lesions are characterized by papules and vesicles while PE lesions are characterized by eczematous plaques. A distinction between CPLE and CAD () may be characterized by a difference in type of lesions (papular and vesiculous versus ←→eczematous resp.), a difference in MED (a normal MED versus an extremely low MED resp.) and possibly by a difference in gender (predominantly women >30 years versus predominantly men >50 years, resp.). ←→

Langerhans cell persistence in the UV-B-exposed skin does not seem to discriminate between different PLE subsets as all PLE patients and even a patient with sub-acute cutaneous lupus erythematoses display this persistence (**chapter 2**). Furthermore, Torres-Alvarez *et al* (1998) demonstrated that UV-resistant Langerhans cells were still present in the skin of patients with actinic prurigo after exposure to 20 MED from a UV-C/UV-B light source. An impairment in UV-induced Langerhans cell depletion might, on the other hand, be used as a diagnostic criterion for photosensitivity of skin disorders because no persistence was observed in healthy volunteers and patients with (non-photosensitive) psoriasis.

FUTURE RESEARCH

Many investigations have been performed on immune parameters in lesional versus non-lesional skin of PLE patients. However, the experiments presented in this thesis give information on the initial phase, not the lesional phase, of a UV-induced skin reaction in PLE patients. Knowledge of this early phase of the pathological skin reaction can give more insight into the pathogenesis of PLE as well as provide some potential tools for early preventive or therapeutic interventions. Therefore, it is important that investigations on the initial effect of UV on the skin of PLE patients are continued. To this end some suggestions will be made for future research.

Besides the studied cytokines, chemokines and chemokine-receptors are important in the trafficking of Langerhans cells. Chemokines, like for instance MIP3- α and MIP3- β , are involved in the influx and efflux respectively of Langerhans cells from the skin. Immature dendritic cells respond to MIP3- α via chemokine-receptor CCR6. Mature dendritic cells, on the other hand, have lost responsiveness to MIP3- α but acquire a responsiveness to MIP3- β via upregulation of the chemokine-receptor CCR7 (Dieu-Nosjean *et al*, 1999). Thus, during migration and maturation Langerhans cells increase the expression of CCR7. In a pilot study we examined the expression of CCR7 in the (un)exposed skin of PLE patients and healthy individuals. Curiously, as the Langerhans cells disappeared the expression of CCR7 in the epidermis increased and was at a maximum 48h post irradiation. No CCR7 expression was observed in the unexposed skin. The expression was slightly less in the UV-B-exposed skin of PLE patients (data not shown). These observations indicate that CCR7 in the UV-B-

exposed skin is not expressed by Langerhans cells and most likely neither by lymphocytes, as hardly any epidermal T-cells could be detected in the UV-B-exposed skin. Which epidermal cells do express CCR7 after UV exposure needs to be determined. Future investigations on the expression of chemokines and chemokine-receptors could perhaps solve this problem and could give more information on the causes of an impaired Langerhans cell migration in PLE patients.

The impairment in UV-B-induced Langerhans cell depletion may not be restricted to PLE patients but could be involved in other photosensitive skin disorders as well. An extensive evaluation of UV-induced Langerhans cell depletion in photosensitive skin disease (i.e. lupus erythematoses and photosensitive eczema) and non-photosensitive skin disorders (i.e. atopic dermatitis and psoriasis) could provide more information on similarities in the pathogenesis. In addition, this could demonstrate whether an impairment in UV-induced Langerhans cell depletion could be used as a diagnostic tool for photosensitivity.

From this perspective it would be interesting to know whether this defect in UV-B-induced Langerhans cell depletion is also observed in PLE patients who only develop a pathological skin reaction to UV-A. If not, this would provide some evidence for a different pathogenesis in UV-A-induced PLE.

It was shown that antigen presentation by Langerhans cells could not take place in the epidermis of the UV-exposed skin of PLE patients. However, an increased number of activated and matured dermal Langerhans cells was observed in the UV-B-exposed skin of PLE patients compared to healthy controls. It needs to be determined whether this is the result of a delayed reaction in PLE patients and whether this higher number of cells may also be found in healthy individuals earlier after UV exposure. A follow-up study identifying Th1 and Th2 cells by expression of specific chemokine-receptors (CCR5, CXCR3 and CCR3, CRTH2 respectively) could give more information on the type of response (type 1 or 2) that evolves in the skin of PLE patients after UV exposure. Furthermore, the expression of activation markers on T-cells, e.g. CD25, should be evaluated to reveal whether these cells are indeed activated.

Regulatory T-cells, which are recognized by the expression of CTLA-4 and the secretion of high levels of IL-10 and TGF- β and low levels of IFN- γ are probably involved in UV-induced immunosuppression (Schwarz *et al*, 2000). It would be interesting to investigate the presence of these regulatory T-cells in the skin of PLE patients in relation to the (impaired) immunosuppression, as shown by van de Pas *et al* (*in press* (*Journal of Investigative Dermatology*)).

In our experiments we looked at the cytokine expression in cytoplasm of cells in the skin *in vivo*. Measuring cytokine levels in a skin suspension by ELISA or in blister fluid of (un)exposed skin could give more quantitative data on the cytokine profile in the skin of PLE patients *versus* healthy controls.

The induction of neo-antigens of unknown identity by UV radiation are probably responsible for immune reactivity in the skin. One could isolate T-cells from the skin and stimulate these cells with a suspension of UV-B-exposed skin (containing the putative neo-antigens). A comparison of T-cell proliferation, T-cell activation and cytokine production in T-cell clones of PLE patients and healthy controls could also give more information on the type of response. An ambitious follow-up study could focus on the identification of these neo-antigen(s) that are specifically responsible for the UV-induced immune reaction in PLE patients.

Because we wanted to reproduce the data observed by Cooper and co-workers and because we could easily detect a difference between PLE patients and healthy controls we irradiated the skin with a single dose of 6 MED. However, when reproducing a pathological skin reaction by photoprovocation the skin is repeatedly exposed to UV-B doses of about 2 MED, depending on the size of the tested skin area (Boonstra *et al*, 2000). A follow-up study using a UV-B exposure regimen comparable to the photo-provocation protocol could give some more insight into the ultimate development of the pathological skin reaction in PLE. One should be aware though that these repeated exposures may give rise to a full-blown pathological reaction. In this case, skin biopsies will be taken from lesional skin which may make it hard to distinguish early events leading up to the pathological reaction from later non-specific inflammatory reactions.

UV radiation, especially UV-A, can induce oxidative damage in the skin (Kielbassa *et al*, 1997). This oxidative damage is involved in the formation of erythema (Dreher and Maibach, 2001; Halliday *et al*, 1999). Future research on mediators involved in erythema, like nitric oxid and prostaglandin E₂, could perhaps give an explanation for the moderately increased susceptibility to sunburn in most PLE patients. This could possibly clarify why the erythema response in PLE patients is enhanced compared to the immunosuppressive response and how this erythema response is dampened after e.g. phototherapy.

Supplementation of dietary fish oil or phototherapy subdues the pathological skin reaction in PLE and increases the MED. It would be interesting to know whether UV-induced immunosuppression, as well as Langerhans cell migration and cytokine expression have also returned to a normal, healthy level after therapy. Knowledge of the immunologic effects of different therapies is of importance for refinement and increasing the effectiveness of therapies.

This thesis describes the first steps in unraveling of the pathogenesis of PLE. The present considerations on possible future research clearly delineate the complexity of the pathogenic mechanisms involved in PLE. Well-targeted experiments are likely to provide us with a good understanding of these mechanisms. Ultimately, such understanding may lead to highly specific and efficient therapies for PLE and other related photodermatoses.

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