Summary and discussion
Polymorphic light eruption (PLE) is the most common idiopathic photodermatosis. From 2002 to 2005, our group was a part of a European consortium, called SUNALL, which investigated PLE in a comprehensive programme consisting of several joint projects. The studies included in this thesis have been focused on epidemiology, pathogenetic mechanisms and diagnostic aspects of the disease. In this final chapter the results of these studies are discussed, and recommendations for future research and implications for clinical practice are presented.

**Epidemiology of PLE**

Epidemiological studies in different countries suggested a latitudinal dependency of PLE prevalence in people with sun reactive skin types I-IV. 1-3 PLE was thought to be more prevalent in countries further from the equator as a result of a stronger loss of UV adaptation during the winter time. However, consistent wide-coverage studies on the prevalence of PLE in relation to latitude had never been performed. In Chapter 2 we describe the results of a survey, which evaluated the prevalence and characteristics of PLE in six European countries (Finland, Germany, the United Kingdom, the Netherlands, France and Greece), covering a large range of geographical latitudes.

In this study among nearly 7000 interviewed European indoor workers, we found a prevalence of PLE of 18%, with a standardised female/male prevalence ratio of 2.3, which is consistent with the results of previous investigation.3-6 There was a strong inverse relationship between the prevalence of PLE and skin phototype. The prevalence decreased from 32% in individuals with skin type I to 9% in individuals with skin type IV-V. Several smaller studies have reported similar results. 3;7-9

The highest prevalence of PLE occurred in the most southern city, i.e. Athens (19.5%, latitude 38°), and the lowest in the most northern city, i.e. Turku (13.6%, latitude 60.5°). In the other European centres the prevalence varied between 17.0% and 18.9%. Moreover, no differences in the proportions of people reporting PLE rashes during springtime were observed between the different countries. Both in Greece and Finland percentages of 10% were found, without any indication of a latitudinal gradient, suggesting that the springtime UV exposure is equally sufficient to trigger the rashes in both countries.

In addition, the occurrence of PLE-rash was higher during sunny holidays (14 to 20%) than during springtime (7-10%), except in Finnish participants, where it was only 9% during sunny holidays. This may explain the overall low prevalence of PLE in Finland. The quality of life due to PLE was reduced most among Greek PLE patients (40% of patients), while in the Netherlands it was reduced in only about 8% of patients.
Our results indicate that PLE is highly prevalent among fair-skinned people in Europe. However, our results argue against the notions that the prevalence of PLE is dependent on a latitudinal gradient and that this skin condition develops most commonly during springtime. Thus, it appears that the prevalence of PLE does not reflect a stronger loss of UV adaptation in temperate climates than in tropical and subtropical climates. Our data rather indicate that holiday sun exposures play a more prominent role in eliciting PLE than sun exposures during springtime in all countries, except Finland. In the present study we did not investigate whether the sunny holidays were spent in the subject’s own country or abroad, and we could therefore not ascertain whether the low prevalence of PLE among the Fins during sunny holidays was attributable to a difference in holiday destinations. Generally, for those living in northern Europe, sunny holidays are holidays abroad, and they gravitate to the Mediterranean.

Therefore, we conclude from our study that (sun-seeking) behaviour during holidays causes PLE more frequently in the general population than factors related to geographical location or early spring exposures. Interestingly, an additional study of ours, not included in this thesis showed that the impairment of quality of life (QOL) in springtime, but not summer, correlated with PLE clinical severity. \(^{10}\) This finding suggests that springtime eruptions tend to occur in the severest cases of PLE, whereas a PLE rash in summer occurs also in milder cases of PLE. Moreover, the low prevalence of PLE during sunny holidays among the Fins suggests that behavioral adjustments can strongly reduce skin eruptions in the milder cases. PLE is most likely prevented or ameliorated by avoiding sudden extensive sun exposures on a non-adapted skin. Instead, the exposures should be gradually incremented in order for the skin to adapt (i.e., attain ‘UV hardening’, see chapter 4). Future studies should elaborate on preventive strategies and severity. We return to these issues in the next sections.

### Pathogenetic mechanisms of PLE

In healthy individuals, UV irradiation induces suppression of cellular immunity, which is associated with an efflux of epidermal Langerhans’ cells (LCs) from the skin and an influx of neutrophils. Previous studies reported that these UV-induced migratory responses of immune cells were reduced in uninvolved skin of patients with PLE. \(^{11;12}\). These observations led to the assumption that PLE could be the result of an imbalance between UV-induced pro-inflammatory and immunosuppressive responses in the skin.

Elaborating on the previous findings, we investigated:

1. Whether the reduced migration of epidermal LCs and neutrophils upon UVB irradiation was specific for PLE or also present in other photosensitive dermatoses, e.g. lupus erythematosus.
2. Whether the supposed imbalance in UV-induced immune cell responses in PLE would normalise after a course of UVB therapy in PLE patients.
3. Whether there are differences in the concentrations of pro-inflammatory cytokines and chemokines involved in the migration of LC and neutrophils upon UVB irradiation between normal skin of PLE patients and skin of healthy controls.

Is reduced Langerhans cell and neutrophil migration also present in photosensitive lupus erythematososis?
In the original study of Köllgen et al. a reduced epidermal depletion of LCs after UV exposure was not only observed in patients with PLE, but also in a patient with photosensitive subacute cutaneous lupus erythematosus (SCLE), who had been included erroneously in that study. 11 This observation might suggest that diminished epidermal LC depletion is not specific for PLE, but a feature shared with other photodermatoses. However, in our study of 22 patients with photosensitive cutaneous lupus erythematosus described in chapter 3, no difference in the decrease of epidermal LC after UVB irradiation exposure was found between LE patients and healthy controls. Also, the number of epidermal infiltrating neutrophils and macrophages increased equally after UVB irradiation in both groups. This indicates that PLE and photosensitive LE (despite an overlap of clinical presentations) do not share a common pathogenesis, and that other mechanisms must be involved in photosensitivity found in LE.

Epidermal Langerhans cells and neutrophils after UVB hardening therapy in PLE
Repeated treatment with low doses of UVB or PUVA in early springtime is widely used in PLE patients to achieve hardening against sun exposure, and may prevent or diminish the severity of PLE in the subsequent summer season. 13;14

In chapter 4 we investigated the effect of UVB hardening of PLE patients on their cell migratory responses after intense UVB exposure. Before hardening therapy epidermal LC depletion and neutrophil influx upon 6 MED irradiation were significantly reduced in PLE patients who had developed a rash upon prior UVB provocation (UVB-P) when compared to controls. However, PLE patients who had not developed a rash upon UVB provocation (UVB-NP) showed less striking differences from controls. The reduced LC and neutrophil migration in UVB-P PLE patients is consistent with the results of previous studies 11;12;15, in which only UVB-P PLE patients had been included.

After UVB hardening therapy, there were no longer significant differences in the cell migratory responses upon UVB irradiation (6 MED) between UVB-P patients, UVB-NP patients and controls. The mechanisms involved in the normalization of cell migratory responses versus inflammatory responses upon UVB hardening are unknown, but these responses are known to be driven by cytokines. It may
therefore be hypothesized that UV hardening establishes a rebalancing of pro-inflammatory and chemotactic cytokines in the local microenvironment (see next section and chapter 4), and that this normalized cytokine profile suppresses PLE.

The observed difference between patients who developed a rash (UVB-P) and patients who did not develop a rash (UVB-NP) upon UVB provocation is intriguing. Remarkably, UVB-P patients did not develop a PLE rash more frequently during hardening with a broadband UVB source than UVB-NP patients. As described in chapter 6, no correlation between the PLE severity score (PLESS) and the results of UV provocations was found. UVB-P was associated with a high score of the severity item ‘number of months affected per year’, suggesting that these patients remain UV-susceptible for a long period of time per year and will not achieve UV hardening easily by natural sun exposure. The normalization of the UV-induced cell migratory responses by repeated very mild UVB exposures in UVB-P patients suggests that a UVB source is more effective to achieve hardening than the UV spectrum of the sun. It could be hypothesized that resistance to UV hardening would be more of a problem than initial susceptibility, and that this resistance represents more truly the severity of PLE as experienced by the patient. A follow-up study to test a correlation between severity of PLE and a resistance to UVB hardening would be highly desirable to gain a better understanding of the disease and for proper preventive interventions.

We have not investigated the effect of UVB hardening in relation to responsiveness to UVB provocation in the present study, and we are unaware of any other study testing this.

**Chemokines and pro-inflammatory cytokines in PLE**

The mechanisms underlying the reduced trafficking of LC from and neutrophils into the epidermis upon UVB irradiation in PLE patients are poorly understood. Chemokines and cytokines are cell recruiting and signaling proteins that are key players in inflammatory responses in the skin and other organs. LC migration from the epidermis is induced and enhanced by cytokines like TNF-α, IL-1α and IL-1β, while IL-8 (CXCL8) and MIP-1α (CCL3) are chemotactic for neutrophils. Differences in the concentrations of these chemokines and cytokines involved in LC and neutrophil migration between PLE and healthy controls might explain the reduced trafficking of LC and neutrophils in PLE patients upon UV irradiation. In Chapter 5 we therefore investigated the concentrations of a selected set of chemotactic and pro-inflammatory cytokines in suction blister fluid raised 16 hours after UVB irradiation in 6 PLE patients and 6 healthy controls. Based on the results described in Chapter 5, only PLE patients who had developed a rash upon prior UVB provocation were selected.
Cytokine levels in unirradiated skin appeared similar in both groups, except for IL-1Ra, which was significantly lower in PLE patients (p<0.05). Although UVB irradiation caused significant increases in IL-1α in both groups, the levels of IL-1α and IL-1β were 2-fold higher in the PLE group than in the control group. Accordingly, the ratios of IL-1Ra over IL-1α and over IL-1β were overall lower in the skin of PLE patients, suggesting that an amplified early pro-inflammatory cytokine response occurs in UVB-irradiated skin of PLE patients.

Our results did however not offer an explanation for the reduced leukocyte migration in PLE patients. Consistent with previous studies IL-8 and TNF-α levels increased strongly upon UVB irradiation, but it was similar in both groups. Aberrant epidermal neutrophil infiltration in PLE patients after UVB irradiation could therefore not be attributed to IL-8, neither to MIP-1α, MIP-1β or MCP-1, as we did not observe any differences in these chemokines between patients and controls. In addition, the results of recent studies demonstrating that peripheral blood neutrophils of PLE patients have a similar chemotactic response to IL-8 as controls argue against reduced expression of IL8 receptors (IL8RA/CXCR1/CD128 and IL8RB/CXCR2/CD182) by neutrophils in PLE patients as an alternative explanation for the reduced migration of neutrophils into the epidermis. With respect to LC, we had expected to find lower levels of IL-1α and IL-1β in UVB irradiated PLE skin, as a possible explanation for the lower LC migration. However, our observations of higher IL-1α, IL-1β levels and similar TNF-α levels in PLE patients compared to controls are seemingly in conflict with lower LC migration. On the other hand, increases in IL-1 may modulate the cytokine profile to cause a shift in types of infiltrating cells: e.g., by release of migration inhibitory factors such as MIF. With our limited set of selected cytokines, we have not screened for such secondary, inhibitory factors. Evidently, follow-up studies are necessary to discover the mechanisms underlying the abnormal LC and neutrophil migration in UVB-exposed skin of PLE patients.

UV provocation testing in PLE

The diagnosis of PLE is often based on a detailed interview with strict inclusion and exclusion criteria (see also chapter 6). In cases with inconclusive patient’s histories, it can be helpful to perform photo provocation tests. Combining broadband UVA with UVB lamps is very successful in inducing PLE lesions, as are solar simulating UV lamps, with 56% to 90% of patients testing positive. With UVB lamps only 35% of patients were found to test positive. In our present study, PLE induction was found in 79% (n= 147) of patients after UVA and in 47% (n= 60) after UVB provocation. In spite of differences in methodologies, our results are similar to those of the aforementioned studies. Besides the spectrum of the UV sources, the number of daily exposures is an important determinant of the success rate of photo provocation (75-85% after 4 days and only small increases with additional exposures), as is the number of
days of observation after the last exposure (prolonged observation after 4 daily exposures without further exposures yielded about the same increases in percentages as with continued daily exposures).\textsuperscript{20}

Since the number of exposures required for a positive response varied among the patients and a substantial percentage (22\% in our study) remained unresponsive after 3 successive daily exposures, it was suggested that PLE lesions would develop most rapidly in patients with severe symptoms of PLE. A recent study had indeed shown a correlation between a severity score and the outcome of provocation testing.\textsuperscript{21} However, this had only been investigated in 9 PLE patients, a group size that we considered far too small to correlate reliably the two widely varying variates for severity and ease of photo provocation of PLE.

In chapter 6, we examined whether the ease of disease provocation by UVA and/or UVB radiation correlates with clinical features of PLE including those indicative of disease severity. We performed provocation testing with broadband UVA and UVB lamps in 143 PLE patients. In addition, a range of clinical characteristics of the disorder, including a 5-item PLE severity score (PLESS), was assessed by a questionnaire. No correlation was found between PLESS and the outcome of UVA and UVB testing. We did, however, find some correlations with items from the PLESS: viz. the number of months that an individual is affected with PLE per year correlated with UVB-P, and facial involvement with UVA-P. Overall, our findings indicate that the provocation results do not correlate with the clinical severity of PLE, unlike the finding of Palmer et al.\textsuperscript{21} Hence, we assert that it is highly dubious to use the photo provocation as a measure of severity. And vice versa, the severity experienced by the patient need not be predictive of the outcome of photo provocation.

**Overall conclusions and future perspective**

**Disease severity**

Based on the results of our hardening and provocation studies (chapter 4 and 6), we conjecture that the ease of photo provocation of PLE at a certain moment in time is not a decisive factor for disease severity, but the resistance to UV hardening is. A lack of hardening would confront the patient with protracted skin reactions during the sunny season, which in the most severe cases would already start in early springtime (as indicated by our result of a significant correlation between PLESS and the impact on quality of life in springtime).\textsuperscript{10} A follow-up study to test the hypothesis of a link between severity of PLE and a resistance to UVB hardening would be highly desirable to gain a better understanding of the disease and to use proper preventive interventions.

**UVB-P versus UVB-NP**

Our study established an intriguing difference between UVB-provoked (UVB-P) patients and other (UVB-NP) patients in UVB-induced migratory responses of
LCs from and neutrophils into the skin. The reduced migratory response in UVB-P patients upon exposure to 3 and 6 MED could not be related to reduced concentrations of chemotactic cytokines in the skin of these patients when compared to healthy controls, but these patients did show a significant bias toward IL1-driven reactions (i.e. low IL1R/IL1α and IL1R/IL1β ratios). This bias could obviously enhance pro-inflammatory reactions and the risk of PLE.

Hardening is known to increase the MED, and therefore lower the susceptibility to UV-induced inflammation (erythema and skin infiltrates, involving COX2 activation and PGE2 release, which can be induced by IL1β). It is conceivable that, rather than boost UV-induced migratory responses of LCs and neutrophils, UV hardening simply corrects the IL1-based pro-inflammatory bias, i.e., the MED increases without any alteration in the UV sensitivity of the cell migratory responses. In our study, the MED after UV hardening was on average increased by only 40%, suggesting that a small imbalance in cytokines is sufficient to trigger PLE. The IL1 bias may be corrected by increased levels of IL1Ra which is indeed found to be elevated in regularly exposed skin. As we have focused on UVB-P patients in search for cytokine differences that could explain the reduced migratory response after a 6-MED exposure, our study unfortunately provided no data on whether UVB-NP patients show an IL-1 bias in their skin. Future studies should certainly pursue this possibility, and investigate whether correction of the IL-1 bias is a common feature of UV hardening in both UVB-P and UVB-NP patients.

UVA-induced PLE
PLE induced by UVA sources remains more enigmatic since it is not yet correlated to such clearly aberrant responses in IL-1 cytokines and migration of LCs and neutrophils as found in UVB-P patients. Because more of the patients are provoked by UVA than UVB testing, it is important to focus in future studies on the pathogenetic mechanism involved in provocation with UVA sources. It is of note, however, that most of the broadband UVA sources used in photo provocation also emit erythemally effective doses of UVB (280-315 nm wavelengths); the Cleo Performance lamps we used emit about 50% of their erythemally effective dose in the UVB band. Hence, UVB radiation may still be an important component of these broadband sources. The fact that provocation with these broadband UVA sources is carried out with a constant dose of 20 kJ/m²/d may also be important to the success rate of provocation, i.e. the dose in MEDs is more variable in UVA testing. In this respect, it could be speculated that UV exposures in MEDs should not be too high in order to observe deviant allergic responses in PLE patients.

Ultraviolet radiation (UVR)-induced expression of pro-inflammatory genes in human keratinocytes is thought to be important for the pathogenesis of photosensitive skin diseases such as polymorphous light eruption. During the acute stages of inflammation, homeostasis is altered, resulting in the cytokine
stimulated (IL-1 and TNF-α) release of ICAM-1 which promotes leukocyte adherence to the endothelium. ICAM-1 is expressed by the vascular endothelium, macrophages and lymphocytes. Norris et al. observed an up-regulation of ICAM-1 in lesional skin of PLE patients. UVA-1 radiation (340-400 nm) induced a reactive oxygen species dependent ICAM-1 gene expression in normal human keratinocytes, and the AP-2 binding site of the ICAM-1 gene promoter region was identified as the UVA radiation responding element. It was therefore hypothesized that a dysregulation at the level of AP-2 or a disturbance within the AP-2 pathway may form the pathogenetic basis of PLE.

Furthermore, UVA-1 induced membrane damage from reactive oxygen species had been surmised to play a role in the pathogenesis of PLE and topical anti-oxidants appeared a successful therapeutic strategy against PLE rash induction.

High UVB dosages were found to impair LC in antigen presentation and T-cell activation, but high UVA1 dosages did not. The UVA-driven induction of ROS may be responsible for the generation of neo-antigens in the vicinity of cell membranes and class I and II major histo-compatibility complexes on LCs, which may be conjectured to initiate an allergy-like response in PLE patients. An insufficiency in UVB-induced immune suppression may be merely an aggravating factor of PLE which is easily overcome by UVB hardening. Patients exclusively susceptible to UVA-1 provocation may then not benefit from UVB hardening at all. Hypotheses like this one need to be tested in future studies to gain insight in the UV(A)-induced pathogenesis of PLE, which is essential to improve preventive interventions and therapies.

**Sense or nonsense of photo provocation of PLE**

Considering the discussion above, we are left with the question of whether photo provocation at present has any merit in clinical practice in diagnosing PLE or providing a prognosis or selecting a preventive strategy. And if so, when should it be used precisely?

Upfront it should be reiterated that photo provocation can be used in case of inconclusive history on a possibly photo(UV)-provoked dermatosis. But in daily practice history taking is commonly very clear. In differential diagnosis with other photodermatoses such as photosensitive lupus erythematosus (serology more important) or chronic actinic dermatosis (low MED) its role appears to be very limited. Moreover, a comparison between UVB and UVA provocations does not - as yet - provide any useful information, except perhaps for selecting proper sunscreens if needed. In all, we must conclude that photo provocation is currently primarily a research tool of clinical experimentation on PLE patients, and it still needs more research to become a really useful clinical tool for diagnosis and prognosis of PLE.

**References**


