Effects of ultraviolet radiation on cutaneous T cells

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Chapter 7

Summary and Discussion
The aim of this thesis was to investigate the fate of the cutaneous T-cell population in situ after a single physiological dose of SSR, UVB or UVA. Small areas on the buttock skin of normal human volunteers were exposed to single doses of UV and punch biopsies were taken at several time points after exposure. The effects of UV on cutaneous T cells, on cutaneous cytokines that are known to regulate T-cell function, and on adhesion molecules expressed by EC that regulate T-cell migration, were assessed by immunohistochemistry and RT-PCR. Additional experiments were performed in vitro to provide a more precise description of the phenotypes and functions of cutaneous T cells isolated from the irradiated site. Overall, we found that a single exposure to UV led to a long-term changes in the number and composition of the cutaneous T cells, in the cytokine production and in the expression of adhesion molecules on EC in the irradiated skin. Because T cells and cytokines play pivotal roles in the regulation of cutaneous immune responses, we speculated that such changes might have a biologically relevant role in UV-induced immunosuppression.

1. Effects of UV on the intraepidermal T-cell populations.

In the studies described in this thesis, we consistently found few lymphocytes in the epidermal compartment of non-lesional, clinically normal skin. In line with previous reports (1-3), they were mainly of the CD8 subpopulation bearing an αβ TCR. More than 60% of the total intraepidermal CD3+ T cells were of the memory/effector phenotype. As we previously pointed out (see Introduction), the expression of markers that indicate T-cell activation on the intraepidermal T cells is still controversial. Our finding that about 70% of the total CD3+ intraepidermal T cells did not express any markers of recent or late activation is in accordance with a previous study by Foster et al. (3), in which intraepidermal T cells were regarded as being in a “resting state”. Resting T cells might secrete a discrete subset of cytokines upon stimulation, such as IFN-γ (type 1 T cells) or IL-4 (type 2 T cells), and might in this way determine the outcome of the cutaneous immune responses. The nature (type 1 or type 2) of the resident epidermal T-cell populations in normal human skin is still unknown. In our studies, we could detect only a very low signal for IL-4 mRNA and no IL-4 protein in normal human skin, but we did find some IFN-γ+ T cells. These findings are in line with the findings of other researchers (4-6), and taken together, they might indicate that the distribution over type 1 / type 2 T cells in normal human skin is dominated by the type 1 T-cell subset.
Studies in vitro have shown that the viability of T cells is hypersensitive to relatively low doses of UV radiation compared with that of other cutaneous cell populations such as keratinocytes (7), monocytes (8) or fibroblasts (9). Taking into account that significant quantities of UV radiation transverse the epidermis (10,11) and that T cells are highly susceptible, we hypothesized that exposure of skin to physiological doses of UV radiation could kill T cells residing in the epidermis (7). Indeed, we found that the numbers of the intraepidermal T-cell populations were significantly reduced in the first few days after a single physiological dose of SSR, UVB or UVA. However the mechanism by which UV irradiation induces intraepidermal T cells depletion in normal human skin is still obscure. The most likely explanation for this loss of T cells is that they are depleted due to phototoxic effects of UV radiation, although, as an unlikely alternative, migration of T cells out of the epidermis can not entirely be excluded. A decrease in intraepidermal T-cell numbers upon UV exposure is not specific for normal human skin. A similar effect of UV radiation on the intraepidermal T-cell population in lesional skin of psoriatic patients has been found after treatment with UVB (12-15). Successful UVB phototherapy of psoriatic patients induced a reduction in the number of epidermis-infiltrating T cells that are thought to contribute to the pathogenesis of the disease. The mechanism of this reduction is most likely cell death through apoptosis (15), which might also account for the intraepidermal T-cell depletion found in normal human skin.

Recently, it has been speculated that the increased susceptibility towards UV-induced apoptosis of T cells may be considered to be a protective mechanism towards such environmental trauma (16). UV-damaged T cells are no longer needed and they could become dangerous. Activation of signalling pathways (e.g. FAS/FAS ligand) eventually leading to apoptosis may facilitate the elimination of such cells and may protect the skin from autoimmune reactions. On the other hand, the undesirable consequences of this physiological protective response to UV is the disruption of a tumor suppressor and infection suppressor mechanism that might be represented by the subset of intarepidermal T cells that produce IFN-γ. This possibility provides an additional mechanism that may account for the occurrence of skin cancers and exacerbation of skin infectious diseases upon exposure to sunlight.

2. Effects of UV on the dermal T-cell populations.

2.1. Mechanism of the UV-induced influx of T cells in normal human skin.
In contrast to the epidermal data, the level of perivascular dermal T-cell numbers showed an UV-dose-dependent increase instead of a decrease during the first few days after SSR and UVB irradiation. The numbers of dermal CD3$^+$ T cells were increased at 24 h, were maximal at 48 h, and progressively dwindled thereafter. The CD3$^+$ T-cell population can roughly be divided into CD4$^+$ and CD8$^+$ subpopulations. Our studies revealed that the UV-induced inflammatory response in the skin in vivo was characterized by selective accumulation of CD4$^+$ but not CD8$^+$ T cells. Because only 2% of the UV-induced dermal T cells expressed the proliferation marker Ki-67, we reasoned that accumulation of these cells was due to influx of T cells from the blood stream.

Modulation of adhesion molecules on EC is essential in regulating the localization, timing, nature, and progression of T-lymphocyte accumulation in inflammatory responses. In the studies described in this thesis, we studied the integrin expression on the UV-induced infiltrating T cells, and related this to the effects of UV exposure on counterreceptors on dermal EC. Apparently the pathways E-selectin/CLA and VCAM-1/VLA-4 are not essentially involved in the recruitment of the majority of the dermis-infiltrating T cells. Since the kinetics of the number of dermal LFA-1$^+$ T cells correlated with the kinetics of the number of ICAM-1$^+$ EC upon UV exposure, it is likely that the recruitment is mediated by LFA-1/ICAM-1 interaction. However, to validate this hypothesis functional studies are needed. Furthermore, since only 47% of the UV-induced dermal T cells expressed LFA-1, it is likely that other receptor/ligand pairs may be involved in this process. In a recent study (17), a newly identified molecule designated lymphocyte endothelial-epithelial cell adhesion molecule (LEEP-CAM) has been shown to mediate T-cell adhesion to EC. It would be of interest to investigate the role of this or of others adhesion molecules, such as P-selectin, in the recruitment of T cells into the UV-irradiated site.

Cytokines play a key role in modulating the expression of adhesion molecules on EC. Exposure to UV triggers keratinocytes and mast cells to produce and/or release IL-1 and TNF-α. Studies on the kinetics of release of UV-induced cytokines in human skin in vivo have indicated that IL-1 (18) and TNF-α (19) are late mediators in UV-induced inflammation. In our studies we showed that T cells were recruited in the late phase of UV-induced inflammation. These two cytokines may play a role in T-cell recruitment into UV-irradiated site. However, studies in vitro have shown that IL-1 and TNF-α can each increase the adhesiveness of EC for T cell (20,21) and neutrophils (22-24); thus, they do not specifically
promote recruitment of CD4+ T cells. In contrast, IL-4 increases the adhesiveness of EC for T cells but not for neutrophils (25). Furthermore, in vivo cytokines are more likely to act in combination at sites of inflammation. Interestingly, it has been shown that the simultaneous presence of IL-4 and TNF-α selectively enhanced EC adhesiveness for T cells (26). In our studies, we found that UV-exposure induced the expression of IL-4 in CD11b+CD15+ cells in normal human skin. This may be of key importance in determining the dominant recruitment of T lymphocytes into the UV-exposed skin. IL-6, which is upregulated upon UV exposure (27), might further amplify the recruitment of T lymphocytes into the irradiated skin. Indeed, it has been shown that IL-6 acted on human EC increasing their adhesiveness preferentially for T lymphocytes, but not for monocytes or neutrophils (28).

The recirculation of T cells relies not only on the expression of specific adhesion molecules on EC, but also on specific chemokines present in the microenvironment. The chemokine(s) regulating T-cell accumulation into UV-inflamed skin site is(are) unknown. A possible role for the chemokine psoriasin in determining the accumulation and retention of T cells in the UV-irradiated site is supported by the results of our studies. Psoriasin is a newly described low-molecular-mass calcium-binding protein that is synthesized (albeit at low levels), and partially secreted by noncultured unfractionated keratinocytes from normal human skin (29). Additionally, functional studies in vitro showed that psoriasin preferentially attracts the CD4+ T-cell subset, whereas it does not attract CD8+ T cells (30). Here, we found in vivo that the expression of psoriasin was strongly upregulated both at mRNA level and at protein level in UVB-exposed skin for a prolonged time (1 to 10 days after exposure). The psoriasin protein was predominantly expressed in the deep follicular epithelium at day 2 after exposure, but at day 10 it was highly expressed in the granular layer of the interfollicular epidermis. The close correlation between these skin sites and the location of the accumulated CD4+ T cells over the time course (first in the dermal compartment and then in the epidermal compartment) suggests that psoriasin may play a key role in the infiltration of CD4+ T cells into UV-irradiated skin. Such a possible role of psoriasin as chemoattractant for UV-induced T cells implies the presence of a psoriasin receptor on the recruited CD4+ T cells. So far, the absence of specific mAb against the psoriasin receptor makes it impossible to investigate this. A T cell is likely to encounter several chemokine gradients as it moves in a given microenvironment. Also other chemoattractant factors, e.g. IL-8, are released into irradiated skin (19). However, IL-8 is chemotactic for both CD4 and CD8 T-cell subsets (31).
Interestingly, although IL-4 itself is not chemotactic for T lymphocytes, it inhibits only CD8\(^+\) but not CD4\(^+\) T-cell chemotaxis towards IL-8 (31). Because in addition to the expression of IL-8, the expression of IL-4 is also induced by UV-exposure, this provides another explanation for the selective accumulation of CD4\(^+\) T cells in irradiated skin.

2.2. Speculations on the biological role of the UV-induced recruitment of T cells in the dermal compartment.

Because T cells play a dominant role in the regulation of immune responses and because it is generally believed that the T-cell phenotype is related to its function, we reasoned that our understanding of the biological role of UV-induced T-cell recruitment would be partially explained by investigating the distinctive cell-surface phenotypes of these T cells. We found that the UV-induced T cells were almost exclusively of the memory CD4\(^+\) phenotype, and the majority expressed an α/β TCR. Because many cutaneous inflammatory disorders (32,33), but also a mild injury such as a suction blister (34), show infiltrates dominated by memory CD4\(^+\) T cells, the UV-induced migration of memory CD4\(^+\) T cells into the skin is likely not to be specific (antigen-independent cutaneous inflammation). On the other hand, as an unlikely alternative, given their memory phenotypes, one might speculate that the UV-induced T cells may be involved in the recognition of neo-antigens induced by UV-exposure (antigen-dependent cutaneous inflammation). In this respect, it might be relevant to investigate whether or not the TCR repertoire of infiltrating T cells in the UV-exposed site is restricted and whether these cells expand by a given antigen stimulation. However, acute UV injury is inflammatory and immunomodulatory, and these two are integrally tied together (35,36).

T cells entering the dermis from the microvasculature in UV-exposed skin may reasonably be expected to encounter cutaneous APCs, such as infiltrated UV-induced macrophages or UV-exposed LC; at the same time they might be exposed to a UV-modulated cytokine microenvironment. Presentation of antigens to T cells under these circumstances could result in activation of suppressor pathways such as inhibition of type 1 T-cell responses which will promote the development of type 2 T-cell responses. Although cutaneous APCs in the irradiated site have been suggested to play a major role in inducing preferential activation of type 2 T cells (37-39), the cytokine environment may also represent a major variable that influences T-cell skewing. In the studies presented in this thesis we found that the UV-
induced T cells (which apparently are not committed when they enter the irradiated site) showed a skewing towards type 2 cytokine production in primary cultures, that is only when dermal cells from UV-exposed skin were present, but not with nonirradiated control dermal cells. Although we did not test for it, one may, we think, assume that the dermal cell suspension from UV-exposed skin contained the CD11b+CD15+IL-4+ cells that we observed in vivo, as well as UV-induced macrophages that produce a large amount of IL-10 (40). We speculate that the locally produced IL-4 and IL-10, which are both known to skew T-cell responses towards type 2, may contribute to UV-induced immunosuppression.

Regardless of the mechanism involved in the T-cell recruitment into UV-irradiated human skin, this phenomenon was strikingly different from the response of dermal T cell populations in mice after acute UV exposure. Upon a single exposure to UV, dermal T cells (normally present within mouse skin) showed a rapid reduction in cell numbers which reached a nadir between day 1 and day 2, after which recovery occurred 3 days after exposure (41). The difference between the responses of mice and human dermal T cells upon UV exposure might suggest that in mice circulating T cells preferentially migrate into lymph nodes upon UV exposure (42), while in humans UV exposure apparently redistributes circulating T cells partially into the dermis. Bearing in mind that the "dermal perivascular unit" is a site of immunological reactivity (43,44), recruitment of T cells into this compartment might be biologically relevant. In this respect Butcher and Picker (45) proposed an important role for competitive niche homing (e.g. to the lymph node vs the dermal perivascular unit) in controlling lymphocyte homeostasis and in shaping the immune response. Thus, in humans, recruitment of T cells into UVB-exposed skin might create an additional adaptive immunological compartment that is not present in nocturnal animals such as mice.

3. Concluding remarks

The studies presented in this thesis have provided the first evidence, as far as we know, for reduction in the number of intraepidermal T cells upon UV-exposure in vivo; concurrently, the dermis of the irradiated skin proved to become infiltrated with non-activated CD4+ memory T cells. We believe that the UV-induced disappearance of intraepidermal T cells and the UV-induced accumulation of T cells into the irradiated skin may represent an additional mechanism by which UV mediates immunosuppression. In spite of the results
provided here, several questions remain less than fully answered: i) Is apoptosis the mechanism by which UV radiation induces disappearance of intraepidermal T cells? ii) In addition to psoriasin and LFA-1/ICAM-1, which other chemokine(s) or adhesion molecules is (are) involved in T-cell recruitment upon UV exposure? iii) What is the function of infiltrating T cells and how does this lead to impaired immune response? iv) What is the impact of the cytokine microenvironment in the dermis of UV-exposed skin on the function of the infiltrating T cells? v) What is the identity of the UV-induced CD11b<sup>+</sup>CD15<sup>+</sup>IL-4<sup>+</sup> cell? vi) And finally what are the effects of repeated UV exposures on cutaneous T cells?


Summary and Discussion


