Effects of ultraviolet radiation on cutaneous T cells

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Chapter 1

Introduction
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1. Photoimmunology and Photoimmunodermatology

Photoimmunology is an area of biomedical research that investigates the effects of ultraviolet (UV) radiation (200-400 nm) on the immune system. Interest in this area expanded in the mid-1970’s, when the immunosuppressive effect of UV was demonstrated in animal studies of UV carcinogenesis (1). Induction of skin tumors by repetitive exposure to UVB (280-320 nm) proved to be due to a combination of genotoxic effects and a decrease in immunosurveillance against the tumor cells. Along with these findings, studies on the function of Langerhans cells (LC), which are the primary antigen-processing and antigen-presenting cells of the human epidermis, also provided evidence that UV has immunosuppressive effects (2). Studies on the use of blood and blood-product transfusions and on transplantation of both solid organs and several types of cellular transplants corroborated and expanded the knowledge in this field (3-5). Nowadays, photoimmunology attracts the attention mainly of photobiologists, immunologists, hematologists and dermatologists.

Photoimmunodermatology is a specialised subdiscipline that describes the effects of UV on the skin immune system (SIS), which is defined as the complexity of immune response-associated cells and humoral factors present in normal human skin (6). Appreciation of the role of the skin as an active part of the immune system is relatively recent and UV-induced immunosuppression is a phenomenon that offers a fascinating opportunity to unravel the complex processes of the immune responses in the skin. Interest in this area of experimental dermatology is not a mere academic exercise: it is important because of its potential implications for human health. In this regard, the decrease in immunosurveillance against tumor cells is suspected of having a major role in the development of human skin cancer (7). Indeed, studies with skin-cancer patients suggested that the immunosuppression of contact hypersensitivity (CH) induced by UV-exposure is a risk factor for development of skin-cancer in humans, because 90% of patients with non-melanoma skin cancers (8) and 100% of patients with melanomas (9) were shown to be susceptible to UVB-induced immunosuppression. Since the first observation, the association between skin cancer and UV-induced immunosuppression has been the leading driving force behind the efforts of many investigators to unravel the mechanisms by which UV affects skin immune responses. However, exposure to sunlight and to artificial sources of UV may also have other implications for human health. Intense UV exposure may exacerbate skin infectious diseases
Abnormal cutaneous reactivity to UV is a feature of a group of diseases called photodermatoses (13). Moreover, modulation of the SIS by UV is the basis of phototherapy of various dermatological disorders, such as psoriasis and atopic dermatitis, which are characterised by hyperactivity of the SIS (14,15).

2. Experimental evidence of immunosuppression by UV in humans.

A CH response is a T-cell mediated inflammatory reaction which can be induced by epicutaneous application of an allergenic chemical hapten. It is presumed to be prototypical of immune responses generated against neoantigens expressed by cutaneous malignancies and by infected skin cells. The pathophysiology of CH consists of two different phases: the sensitization phase and the elicitation phase. The sensitization phase is referred to as induction phase or afferent phase and occurs at the first contact of the skin with the hapten. This contact has no clinical consequences, except in some cases, in which the first application of a sensitizing dose of a hapten results in an intense inflammatory response that appears at the application site within 8 to 14 days (primary allergic reaction). In most cases, the second and subsequent contact of the skin with the allergenic hapten lead to development of a reproducible and characteristic inflammatory response (elicitation phase, also known as the challenge phase or the efferent phase of CH).

In recent years, many studies have addressed the effects of UV-exposure on CH in humans (16). Interest in this area of research is based on the speculation that tumor antigens may, like allergenic haptens, be inappropriately presented through UV-exposed skin, resulting in down-regulatory mechanisms that inhibit immune responses against the incipient tumor and allow it to grow (17). Exposing human skin to UV has profound effects on the induction of CH to the contact sensitizer 2,4 dinitrochlorobenzene (DNCB). When a universally sensitizing dose of DNCB was directly painted onto acute UVB-exposed skin of healthy human volunteers within one hour after the last exposure, CH failed to develop in some (40%), whereas other subjects (60%) developed CH (8). The ability of UVB irradiation to impair induction of CH to DNCB in humans has also been reported by Cooper et al. (18). Furthermore, these two studies have shown that a subset of healthy volunteers who failed to develop CH when hapten was encountered through UVB-exposed skin, also became unable to generate a contact sensitivity response after repeated sensitization. In other words, a state of tolerance was induced. In addition, these and other studies revealed that UVB-exposed skin
fails to develop both the primary allergic reaction (8,18-20) and the elicitation reaction (21). Thus, in humans, UVB-exposure is deleterious both for the afferent and for the efferent phases of CH. In addition to the well-established immunosuppressive effects of UVB, recent studies reported a similar effect on CH in humans after a single exposure to different UV sources, such as solar-simulated radiation (SSR) (290-400 nm) (22-24) and UVA-II (320-340 nm) (25). However, exposure to UV does not always lead to immunosuppression. A recent study showed that UVA-I (340-400 nm) irradiation did not affect the immunisation rate in normal human volunteers (26). Moreover, Tie et al. (21) found that subjects who were sensitized through normal skin and challenged via UVB-exposed skin exhibited enhanced CH. Thus, UV-exposure may have suppressive or enhancing effects on the expression of CH in humans, depending on wavelength and sensitization protocol.

Various speculations on the physiological role of UV-induced immunosuppression in human health have been made by several investigators. One generally accepted hypothesis suggests two sides of the coin (27-31). On the one side, the teleological hypothesis that UV-induced suppression of T-cell mediated immunity may have arisen in the course of evolution as a way to protect the organism’s integrity against autoimmune responses that are induced as a consequence of UV-mediated skin injury. In this view, photodermatoses, such as polymorphous light eruption, have been suggested as models of impaired UV-induced immunosuppression (31). On the other side, the hypothesis that the undesirable consequences of this physiological protective response to UV is observed both in occurrence of skin cancers and in exacerbation of skin infectious diseases.

3. Cellular targets of UV: emphasis on cutaneous T cells.

Exposure to UV induces a number of changes in the cellular and humoral components of the SIS, which are crucial for the generation of UV-induced immunosuppression (32-36). The aim of this chapter is to review the changes in human cutaneous T-cell populations upon exposure to physiological doses of UV. The first part focuses on effects of UV on cutaneous and circulating T cells in vivo and in vitro. The second part concentrates on the role of Langerhans cells, macrophages, endothelial cells (EC), keratinocytes, mast cells and neutrophils in cutaneous immune/inflammatory responses to UV in humans, in particular on the ability of these cells to modulate both functional properties and recirculation of cutaneous
T cells.

3.1. Effects of UV on T cells.

3.1.1. Distribution and characteristics of T cells in normal human skin.

Since the 1950's, it has been known that some lymphocytes are localized in the epidermal compartment of nonlesional, clinically normal human skin (37). In the past few decades, advances in cellular and molecular immunology have allowed us to reveal that virtually all the intraepidermal lymphocytes are T cells (38) and to describe the heterogeneous phenotype of these cells (39). Intraepidermal T cells account for approximately 2% of the total number of cutaneous CD3+ cells (38). The vast majority of intraepidermal T cells express the T-cell receptor (TCR) αβ, only a minor portion (1-15%) carries the TCR γδ (40-46). The intraepidermal T-cell population is primarily represented by single positive CD4 or CD8 cells, with skewing towards the CD8 subset (38,42,47). Rare double negative (DN; CD4+CD8+) T cells, mostly carrying the TCR γδ, have been observed (46,48). The vast majority of intraepidermal T lymphocytes express the memory/effector phenotype defined by expression of CD45RO and lack of CD45RA (42,46,49,50). The expression of markers which indicate recent activation, such as interleukin-2 receptor α (IL-2Rα) and HLA-DR, is still controversial. In one study, the majority of intraepidermal T cells lacked IL-2Rα and HLA-DR and therefore these T lymphocytes were regarded as being in a “resting state” (42). In two other studies, these two markers of recent activation were found in a large proportion of intraepidermal T cells (38,46). However, data from different research groups showed consistently that only a few intraepidermal T lymphocytes expressed the HNK-1 epitope (CD57) and the very late activation antigen-1 (VLA-1), both markers that appear late after activation (42,46). While some T cells may migrate into normal skin as “passenger leukocytes”, it is possible that many localize in the epidermis as “resident cells”. However, the biological role(s) of such resident epidermal T-cell populations is(are) still unknown. The predominance of memory T lymphocytes over naïve T lymphocytes might reflect the chronicity of antigenic exposure challenging this tissue and suggests a specific role for T-cell mediated immunoresponses in reply to this challenge. It has been hypothesized that the intraepidermal T cell populations may play a role in continuous immunosurveillance against the development of cutaneous cancers and persistent infection with intracellular pathogens (51,52). Szabo et al. recently demonstrated that human intraepidermal T cells produce
interferon (IFN)-γ (53), which forms the basis of a tumor-suppressor mechanism (54), thus corroborating the above-mentioned hypothesis. Furthermore, given the cytotoxic potential of TCR γδ+ DN (CD4−CD8+) T cells and TCR αβ+ CD8+ T cells, these two cell populations would be good candidates for the role of effector cells in the postulated intraepidermal immunosurveillance. Thus, activated intraepidermal T cell subpopulations may injure antigen-bearing target cells (e.g., keratinocytes, melanocytes) by either the release of cytokines, or MHC-restricted cytotoxicity.

Dermal T cells are preferentially (90%) clustered around postcapillary venules of the superficial plexus, and within the connective tissue sheaths of adnexal appendages (38,49); the remaining 8% are “free” in the connective tissue of the reticular dermis (38). The dermal T-cell population consists of CD4+CD8− and CD4−CD8+ cells; in the perivascular compartment either an equal distribution is seen (38,44,55,56) or preference for the CD4+ subset (49), and “free” in the papillary and reticular dermis a preference for the CD8+ subset (38). Only few dermal DN CD4−CD8+ T cells have been observed (48). The majority of dermal T cells (90%) express the TCR αβ, and only a minor portion (7-9%) carries the TCR γδ (40,43-45). Most dermal T cells belong to the CD45RO+ memory population (38,49,50). The memory phenotype and close apposition to either macrophages (57) or endothelial cells (38), which might induce and regulate cytolytic T-cell differentiation (58), suggest that dermal T cells might be involved in a response against exogenous and/or endogenous antigens. Thus, dermal T cells as well as epidermal T cells may perform a local sentinel function by ensuring rapid responses upon encountering cutaneous recall antigens.

Renal-transplant recipients provide indirect evidence for the role of cutaneous T cells in the immunosurveillance against skin cancer and skin infections. These patients, who usually receive long-term immunosuppressive therapy in order to maintain the viability of the transplanted kidney, have an increased risk of getting skin tumors, such as squamous cell carcinoma (59,60), and of viral opportunistic skin infections such as warts (59). In a recent study (61), it was found that skin specimens from renal-transplant recipients, who did not have any skin diseases, had fewer T cells in both the epidermal and the dermal compartments than age-matched healthy volunteers. Moreover, in situ the depletion of cutaneous T cells increased during the entire duration of the immunosuppressive therapy, which is in line with the clinical evidence that the longer the immunosuppression is present, the more skin cancers and skin infections develop (60).
3.1.2. *In situ* effects of UV on cutaneous T-cell populations.

The appearance of a dermal inflammatory cell infiltrate, mainly composed of T lymphocytes, after exposure to a single dose of either UVB or UVA has been well documented (62-67). As observed in biopsy specimens stained with hematoxylin and eosin, dermal lymphocytic infiltrates, mainly located in the perivascular area, were present from day 1 up to day 3 after UVB-exposure, and declined by day 7. A single exposure to erythematogenic doses of UVA elicited a dermal inflammatory infiltrate indistinguishable in cell type and location from that described for the UVB-exposed skin specimens, but the former was greater in terms of both quantity and depth of cell infiltrate than the latter (62,63). More recently, Norris et al. (68) and Van der Vleuten et al. (69) observed an increase in the number of perivascular CD3+ or CD2+ T cells, respectively, up to 1 day after a single exposure to UVB and both group saw progressive dwindling thereafter. Margolis et al. (70) and Lavker et al. (71) found that the majority of the UVA-induced perivascular mononuclear cells were CD3+ T cells, mostly of the CD4+ subpopulation (70). Lavker et al. (71) observed that repeated exposure to suberythematogenic doses of either UVA or SSR given once per day for 28 days also induced an increase in inflammatory cells, mainly lymphocytes, primarily in a perivenular location. The UVA exposures resulted in significantly more cellularity than the SSR exposure. Similar results were obtained in another study (72), in which exposure to either broad UVA or UVA-I was repeated at 24h intervals for 8 days, resulting in perivascular accumulation of T lymphocytes. The degree of cellularity was directly related to the daily UVA doses.

Upon exposure to UV a relevant quota of incident light reaches the basal layer of the epidermis (73). It has been speculated that intraepidermal T cells, which are mainly located in the basal layer, could easily be killed upon exposure to physiological doses of UV (74). However, so far, no attempt has been made to describe quantitative and qualitative alterations *in situ* of the intraepidermal T-cell populations upon single UV-exposure.

3.1.3. *In vivo* effects of UV on circulating T cells.

In humans, the effects of single and repetitive whole-body exposures to UV on peripheral blood lymphocytes have been investigated in many studies, and were reviewed by Morison (75-77). With the exception of two reports (78,79), changes in circulating T-cell subsets have been consistently found. A single exposure to UVB induced a dose-dependent
decrease in the proportion of circulating T cells (75) and significant changes of the CD4/CD8 ratio (80). Repetitive exposure to the mid-day sunlight (1h per day for 12 days) was followed by a significant decrease in the number of circulating CD3\(^+\) T cells (81). The CD4/CD8 ratio was also reduced, due to a significant increase in circulating CD8\(^+\) T cells and a decrease in circulating CD4\(^+\) T cells. Moreover, subjects exposed repetitively to UV (mainly UVA-I) in a commercial solarium showed a reduction in the numbers of circulating CD3\(^+\) and CD4\(^+\) T cells as well as in the CD4/CD8 ratios (82,83).

The mechanism by which in vivo exposure to either SSR, UVB or UVA-I alters the proportion of circulating T lymphocytes is unknown. It has been suggested that the decrease in peripheral CD3\(^+\) and CD4\(^+\) T cells might be due to migration into lymphoid tissue at other body sites (81). As described in the previous paragraphs, exposure to either SSR, UVB or UVA-I induces accumulation of CD3\(^+\) T cells, likely of the CD4\(^+\) subset, into the dermal perivascular area, which suggests that redistribution of specific subpopulations of circulating T cells into the skin compartment might contribute to changes in circulating T-cell subsets. Interestingly, repetitive exposure to incremental doses of narrow-band UVB (311 nm) given three times per week for 4 to 5 weeks did not induce either perivascular accumulation of T lymphocytes (84) or changes in proportion of circulating T cells (85,86). This is in contrast with all the other studies but may indicate that changes in circulating and cutaneous T cells upon UV-exposure depend on the applied wavelength.

3.1.4. In vitro effects of UV on viability of T-cell subsets.

A large number of investigations have been performed concerning the effects of UV on human T cells in vitro, excluding effects mediated via other cells. Early studies, reviewed by Morison (76,77,87) consistently found a dose-dependent decrease in viability (80,87-92). The sensitivity of T cells to UV also proved to be dependent on the applied wavelength (87,90-92); UVC (200-280 nm) was more lethal than UVB, which in turn was more lethal than UVA. More recent reports suggest that the UV-induced killing of T cells is due to a combination of effects on different cellular targets, such as DNA and plasma-membrane receptors, activating a cascade of events eventually leading to cell death. Apoptosis is a physiological form of cell death characterised by nuclear condensation and cell shrinkage with preservation of an intact plasma membrane. UVB has been shown to be a particularly potent inducer of apoptosis in peripheral human T cells (93,94). The mechanism is not
entirely clear, but may involve DNA damage (93), and alteration of the Fas/FasL signalling pathway (95).

The reports concerning the effects of UV on viability of CD4^+ or CD8^+ T-cell subsets are controversial. In one study, the investigators found that CD8^+ T cells were more sensitive to UVB than CD4^+ T cells (80). In contrast, two other studies showed that the CD4/CD8 ratio was not altered upon SSR (96) or UVB (74), indicating that UV had no selective effect on either of these two T-cell subsets. Human activated T cells may also be roughly divided into two polarized subsets (i.e. type-1 and type-2 T cells) based on the pattern of cytokine they secrete, on the immune response they participate in, and on the preferential expression of some surface antigens (97,98). Type-1 T cells produce predominantly IFN-γ, which activates cytotoxic functions of effector cells such as macrophages and CD8^+ cytotoxic T cells. In contrast, type-2 T cells are characterized by predominant production of IL-4, which inhibits several macrophage functions and promotes humoral immunity. Upon UVB-exposure *in vitro* T-cell production of cytokines such as IL-2, IL-4, IL-5, IFN-γ and TNF-α, was reduced in an identical dose-dependent way for all cytokines tested (74). Moreover, a strong correlation was found between loss of viability and the reduction in cytokine productions, which suggests that the fall in production of cytokines was due to cell death.

3.2. Impact of UV on the cellular components of the SIS: effects on functions and dynamics of cutaneous T cells as end-points.

3.2.1. Upon UV-exposure cutaneous antigen presenting cells (APCs) induce preferential activation of type-2 T-cell responses.

Cutaneous APCs, such as LC and macrophages, may have a central role in determining the differentiation of T-cell subtypes and the cytokine-production pattern of the activated T cells in the skin. This T-cell stimulatory function of APCs can be affected by UV radiation, and may ultimately result in a different T-cell response.

The effects of UV on epidermal LC, the “professional” APC of the human epidermis, have been studied for many years since the first reports in 1981 on the deleterious effects of UV on epidermal LC population in humans (64,99). Exposure to SSR (100), UVB (99) and UVA (66) induces a decrease in the number of LC within human skin. Depletion of LC from the epidermis after UV exposure is probably due to a combination of cell death (101) and
enhanced migration to regional lymph nodes (102). In addition to the effects on LC numbers, UV-exposure induces functional alterations of this cell populations. A common theme found in a number of studies reviewed in (103-105) is the UV-exposed LC activation of type-2 T cells accompanied by the induction of type-1 T-cell tolerance. Because type-1 T cells generally help cell-mediated immune responses in CH and tumor rejection, the suppression of this particular cutaneous immuneresponse fits with the UV-induced immunosuppression described above.

At the nadir of depletion of epidermal CD1a^+ LC after UV exposure, CD1a^+, HLA-DR^+, CD11b^+, CD36^+ epidermal macrophages appear (106). These macrophages are distinct from LC, amongst others, in their high production of IL-10 (107,108) and in their inability to induce early up-regulation of T-cell IL-2Rα (109). The UV-induced macrophages activate CD4^+ CD45RA^+ suppressor inducer T cells that induce maturation of CD8^+ suppressor effector T cells, which in turn suppress T-cell mediated responses (110,111). It is unknown whether UV-induced macrophages, in analogy to UV-exposed LC, will preferentially activate a type 2 T-cell response.

The reversal of APC population from LC into macrophages in the UV-irradiated site and the altered capacity to activate type 1/type-2 T-cell responses have been proposed to be the key cellular mechanisms of UV-induced immunosuppression (33). However, recent studies indicate that LC and epidermal macrophages alone are not responsible for the UV-induced suppression of cell-mediated immunity, although it is likely that they are involved in the process (112).

3.2.2. Microvascular EC are the key gate-keepers for circulating T-cell migration into UV-exposed skin.

EC are essential elements in the recruitment of circulating T cells into sites of cutaneous inflammation. Upon activation, EC express cell-surface proteins and glycoproteins known as cell adhesion molecules (CAM), such as E-selectin, ICAM-1 and VCAM-1, that allow circulating T cells to bind to activated EC. A transient interaction between the cutaneous lymphocyte-associated antigen (CLA) and its ligand E-selectin expressed on dermal EC has been suggested as the first step in the extravasation of skin-homing T cells into a skin inflammatory site (113). Subsequently, the circulating T cells, which express or have
locally been induced to express lymphocyte function-associated antigen (LFA)-1 and/or very late activation antigen (VLA)-4, will more steadily interact with ICAM-1 and/or VCAM-1 on dermal EC (114). In humans, upregulation of the dermal vascular E-selectin and ICAM-1 has been described to occur \textit{in vivo} after a single exposure to UVB (68,115,116) or to UVA (115,117). The extent of infiltration of neutrophils and macrophages into UVB-irradiated sites has been correlated with E-selectin upregulation on dermal EC (68,116,118). However, accumulation of T cells did not correlate with the expression of E-selectin on dermal EC in UVB-exposed sites (68), suggesting that CLA-independent transmigration, possibly involving interaction of LFA-1 with ICAM-1 (113), may recruit T cells into UVB-exposed skin. Furthermore, CLA/E-selectin interaction appeared to be irrelevant \textit{in vitro} in UVA-induced T-cell adhesion to human dermal microvascular EC (119). Several investigators consistently reported that expression of VCAM-1 on dermal EC did not change upon exposure to UV (68,115,116), and suggested that VLA-4/VCAM-1 interaction does not play any role in recruiting circulating T cells into UV-exposed skin.

3.2.3. Keratinocytes and mast cells are the major sources of pro-inflammatory cytokines and chemokines relevant for T-cell recruitment into UV-exposed skin.

Human keratinocytes are known to produce a wide variety of soluble peptide mediators (120,121). These humoral factors, produced locally within the epidermal microenvironment and termed "epidermal cytokines", play an essential role during induction, amplification, and resolution of inflammation and skin immune responses. According to Baker \textit{et al.} (122,123), keratinocytes act principally as pro-inflammatory signal transducers, responding to nonspecific external stimuli with the production of inflammatory cytokines, which influences leukocyte accumulation and subsequent immunological events. Indeed, exposure to UV has been shown to trigger keratinocytes to produce and/or release several pro-inflammatory cytokines, such as IL-1 (124-126), IL-6 (127,128), IL-8 (129) and tumor necrosis factor (TNF)-\(\alpha\) (126,130,131).

In normal human skin, mast cells are preferentially located in the perivascular area of the papillary dermis (132,133). A recent study, using the human leukaemic mast cell line HMC-1, which exhibits a number of phenotypic and functional properties typical for tissue mast cells, has shown that these cells can be induced to express the genes for IL-1, IL-6, IL-8,
TNF-α and to release these cytokines (134). Moreover, mast cells seem to be unable to produce cytokines which support type 1 T-cell responses, such as IL-12 and IFN-γ, whereas they produce cytokines possibly involved in type 2 T-cell responses, such as IL-4 and IL-10. Although such a broad but specific spectrum of cytokine secretion makes the mast cell a candidate for playing an important role in UV-induced immunosuppression, only limited data have been generated on the effect in vivo of UV on human dermal mast cells. In vivo, immediately after a single exposure to UVA or UVB human dermal mast cells degranulate (64-66,135), and release a variety of pre-formed mediators, such as histamine (64,65) and TNF-α (136).

UV-induced cytokines influence lymphocyte accumulation by at least two different mechanisms. First, cytokines modulate expression of adhesion molecules on endothelial cells. There is no doubt about the involvement of IL-1 and TNF-α in EC-mediated recruitment of T lymphocytes into inflammatory sites. In vitro, IL-1 and TNF-α induced a time-dependent increase of adhesion molecules on human EC monolayers (137) and increased EC adhesiveness for T lymphocytes obtained from normal human subjects (138). Moreover, direct intradermal injection of the two cytokines led to similar patterns of expression of adhesion molecules on the dermal vasculature of normal human volunteers, and induced a dermal leukocyte infiltrate (139,140).

Second, T-cell migration through the dermal perivascular area to the target microenvironment, such as epidermis, is driven by chemotactic cytokines (i.e. chemokines). In this respect, IL-8 and IL-15 have been shown to be chemotactic factors for T cells (141,142) and recently Duthie et al. (16) speculated that macrophage-derived IL-15 could play an important role in attracting T cells into UV-exposed sites. However, it is still unknown which specific chemokine(s) is (are) involved in the selective accumulation of CD4+ T cells in the skin in response to UV. Psoriasin is a low-molecular-mass calcium-binding protein that is synthesised (albeit at low levels), and is partially secreted by noncultured unfractionated keratinocytes from normal human skin (143). A recent study indicates that psoriasin is a potent and selective chemotactic protein for CD4+ T cells in vitro (144). Also IL-16, a selective chemoattractant for CD4+ T cells (145), which is stored in a bioactive form in mast cell granules (146), might be released upon UV-exposure and therefore participate in CD4+ T-cell chemoattraction.
3.2.4. Neutrophils participate in the switch in leukocyte infiltration from a predominantly acute neutrophil response to a chronic mononuclear cell response in UV-exposed sites.

Neutrophils are most prominent early in the UV-induced cellular recruitment from the blood stream into UV-irradiated human skin. Dermal neutrophils appear immediately after UV-exposure, with an increase in number up to 14 h and progressive dwindling thereafter (67). E-selectin, IL-8 and TNF-α appear to be involved in the UV-induced recruitment of neutrophils (118). The function of neutrophils in UV-irradiated sites is unclear. The ability of neutrophils to generate several chemokines, such as IL-8, macrophage inflammatory protein (MIP)1-α and MIP-1β (147,148), indicates that they might be involved in leukocyte infiltration and might switch the acute neutrophil response to a chronic mononuclear cell response (i.e. by macrophages and T cells) in the UV-exposed site, a phenomenon observed over time (68).

4. Aims of the Studies

In humans, following exposure to single doses of UV, the appearance of a dermal inflammatory cell infiltrate, mainly composed of T lymphocytes, has been well documented in the past. Since T cells play a pivotal role in cutaneous immunology, particularly in the course of inflammatory skin conditions (149,150), it is surprising that, so far, few data have been generated concerning phenotype, mechanism of recruitment, and functional properties of the UV-induced dermal T cells. The aim of this thesis was to further investigate the effects in situ of a single physiological dose of either SSR, UVB or UVA on the dermal T-cell population. Moreover, we focused on changes in the epidermal T-cell populations. Although UV irradiation has only limited depth penetration in human skin (151), epidermal T lymphocytes might be directly affected upon exposure to physiological doses of UV. In the initial time-course study, described in chapter 2, we locally irradiated healthy human volunteers with graded doses of SSR. Quantitative changes in the total cutaneous CD3+ T-cell population, as well as changes in the CD4+ and CD8+ subsets, were assessed by immunohistochemistry. In chapter 3, in which a similar protocol was used, are described studies to determine in more detail the kinetics of the CD4+ and CD8+ subpopulations, as well as the phenotype (such as memory versus naïve, activated versus resting, or TCRαβ versus TCRγδ) of the T lymphocytes that infiltrated the human skin after a single exposure to
SSR or UVB. In order to gain a better understanding of the mechanism by which UVB induces recruitment of T cells into the irradiated site, we investigated the expression of adhesion molecules on infiltrating T cells and related this to the expression of their counterreceptors on EC (chapter 4). We further assessed the effect of UVB on the two chemokines, psoriasin and IL-16 at mRNA level with RT-PCR techniques and at protein level with immunohistochemistry. To further analyze the characteristics of UV-induced T cells, in the study described in chapter 5 we isolated T-cell clones from irradiated skin and examined their phenotypes with respect to the expression of type-1 versus type-2 markers; the results were compared with those obtained in situ using immunohistochemistry. Effect of UV on the two discriminating cytokines IL-4 and IFN-γ were also investigated. Serendipity led us to discover that UVB-exposure induces the appearance of CD3⁺ IL-4⁺ cells into the irradiated site. Chapter 6 describes a study that was conducted in order to identify this previously unknown UVB-induced cellular source of IL-4.
References


Introduction


