Effects of therapies on cytokine patterns in psoriasis

Piskin, G.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter

General introduction and aims of the thesis
Psoriasis: a clinical entity

Psoriasis is a common skin disease with a prevalence of approximately 2% around the world. It is certainly not a new entity as it had already been described by Hippocrates (460-377 BC). Early in the 19th century, the Viennese dermatologists Hebra and Kaposi defined psoriasis as a separate disease entity.

There are various clinical presentations of psoriasis. The most common type of psoriasis is plaque-type psoriasis which is characterized by erythematous, scaling, elevated and well-defined lesions preferentially located on the extensor parts of the body and the scalp. There are different presentations of plaque-type psoriasis according to the distribution and the size of the lesions. Numerous small, eruptive and coin-like plaques distributed all over the body, sparing palms and soles, are seen in guttate type of psoriasis vulgaris. On the other hand, erythrodermic psoriasis is characterized by a generalized erythema and desquamation. In case of flexural psoriasis, psoriatic plaques are preferentially located on the flexor surfaces such as axillae, submammary region and groins. Palms and soles can be the only manifestation site of psoriasis as well. Pustular psoriasis, which is out of the scope of this thesis, is characterized by pustular lesions. According to its localization, it is divided into localized and generalized forms. In addition to the skin involvement, an inflammatory arthritis can develop by psoriasis patients which can affect peripheral and/or axial joints. Prognosis remains as unpredictable as it was almost 200 years ago when it was described as a separate disease entity. Although it is rarely a life threatening disease, it is impossible to say how long the disease will last, whether a relapse will occur, or for what period of time the patient will remain free from psoriasis. In a recent study it was shown that psoriasis patients have more experiences of stigmatization related to their disease compared to the other skin diseases. This means that these patients feel rejected, flawed, guilty and ashamed because of their disease leading them to major social and psychological difficulties.

Pathogenesis of psoriasis

Histopathology of psoriasis. Psoriasis is characterized by 3 major histological changes: i, Epidermal thickening, parakeratosis and hyperkeratosis; ii, capillary dilatation in dermal papillae; iii, and mononuclear and polymorphic cell infiltration in dermis and epidermis. All these changes contribute to the clinical picture in different ways. Thickening of skin is caused by increased thickness of epidermis while erythema is caused by dilated capillaries in dermal papillae. The characteristic silvery scaling of lesions is related to the hyper- and parakeratosis.
Despite the intensive efforts to reveal the etiopathogenesis of psoriasis, the exact mechanism of the formation of skin lesions is still unknown. Psoriasis is commonly accepted as a chronic inflammatory disease with an abnormal proliferative activity of keratinocytes which is caused by activated type 1 cytokine producing lesional T cells. However, it is not known how and why these cells get activated. T cells appear early in the lesions and cause a general chronic inflammatory status in the lesional area. Many different cell types residing in the skin eventually become activated such as endothelial cells, mast cells and fibroblasts which seem to potentiate type 1 cytokine phenotype and keratinocyte proliferation. The literature on the etiopathogenesis of psoriasis is too broad and detailed to include all of its aspects in this introduction. Because of this, the introduction will be limited to the role of T cells, keratinocytes and neutrophils in psoriatic lesion formation as they are the major focuses of this thesis. First, a brief overview on the genetical aspects of this disease will be discussed.

*Genetics.* There is no doubt that psoriasis has a genetic basis. There is a high degree of concordance of psoriasis in monozygotic twins and the disease tends to cluster in some families. Individuals who carry the major histocompatibility complex class I molecule HLA-Cw6 allele have a 10-20-fold increased risk to develop psoriasis. Although the association between this allele and the development of psoriasis is strong it seems that many other genes contribute to the development and severity of psoriasis. Independent genome-wide scans suggested many psoriasis susceptibility loci as reviewed by Capon et al. However, PSORS1 on chromosome 6p21.3 showed the strongest association with psoriasis as demonstrated by different groups. This locus contains genes coding for HLA-C, corneodesmosin (Cdsn), and alpha-helix coiled-coil rod homolog (HCR) which were found to be expressed at higher levels in psoriatic lesional skin than in normal skin. These changes in diseased skin could explain the epidermal hyperproliferation (Cdsn and HCR) and, probably, mononuclear cell infiltration in the lesion (HLA-C). Recently, a meta-analysis of genome-wide studies on five Caucasian and one Chinese Han populations revealed that in addition to PSORS1, a second locus named PSORS9 on chromosome 4q28-q31, leads to susceptibility to psoriasis both in Northern European and Asian populations. In general, psoriasis is still regarded as a “multifactorial” disease in which various genes and environmental factors are involved in the appearance of lesions and the progress of disease.
Central role of T cells in the psoriatic inflammatory reaction. At the end of the 1970s, a possible role of T cells in the immunopathogenesis of psoriasis was introduced. It appeared that the earliest change in psoriatic lesions is the influx of mononuclear cells into the dermis and epidermis. The studies from the last two decades showed that these mononuclear cells which were identified to be T cells play a major role in the formation of skin lesions. There are many findings which contribute to our present knowledge on the role of T cells in psoriasis:

1. Köbner-initiated and spontaneous eruptions of new psoriatic lesions are associated with the migration of T cells into the epidermis.
2. Intradermal injection of interferon (IFN)-γ (a T cell derived cytokine) can trigger psoriatic lesions in non-lesional skin.
3. Specific T-cell suppressors are effective as psoriasis therapy.
4. T cell clones isolated from psoriatic lesions induce the proliferation of keratinocytes.
5. Bone marrow transplantation from a psoriatic donor caused the development of psoriatic skin lesions in the transplant recipient.
6. In an animal model with severe combined immunodeficient (SCID) mice showed that mice engrafted with symptomless skin developed psoriatic plaques when the grafts were injected with blood derived T cells.

In the early 1980s, T cells in the dermis and epidermis of skin lesions were identified as being CD45RO+ memory T cells. In addition, these cells express activation markers CD69 and CD25 showing that they are activated T cells.

Although not as strict as in mouse models, human T cells are roughly divided into two major groups according to their cytokine expression: Type 1 and type 2 cytokine expressing T cells. Both CD4+ helper and CD8+ cytotoxic T cells can show this distinction at different degrees. Because of this reason, we prefer to use the terms “type 1 T cells” and “type 2 T cells”, instead of Th1 and Th2 T cells, which only refer to the CD4+ T cells. Although numerous cytokines were described to be expressed by type 1 (i.e. IFN-γ and tumor necrosis factor (TNF)-β), type 2 (i.e. interleukin (IL)-4 and IL-5) or both (i.e. IL-3, TNF-α and granulocyte macrophage-colony stimulating factor) cell types, IFN-γ and IL-4 are used in most studies as exclusive markers of type 1 and type 2 T cells, respectively. This distinction seems to be important as type 1 T cells are mostly involved in the defence against intracellular microorganisms and tumor cells, while type 2 T cells play a major role in the clearance of extracellular microorganisms such as helminths. The balance between these two immune responses is important. Preference to mount type 1 or type 2 cytokine-mediated immune response can determine the clinical expression of a disease. A good example is leprosy...
where the clinical picture and prognosis can differ in great extent depending on the development of type 1 or type 2 immune response. Similarly, the disturbance of this dichotomy was demonstrated to be important in the development of various immune-mediated diseases. As concerns psoriasis, most investigators agree on the predominance of type 1 cytokine expression in peripheral blood cells and skin lesions in this disease. The major type 1 cytokine, IFN-γ, is expressed in high amounts in psoriatic skin and is demonstrated to contribute to the major histological finding of psoriasis which is the hyperproliferation of keratinocytes (discussed in detail in one of the next sections with the heading *Disturbance of epidermal cell homeostasis in psoriatic skin*).

Regulation of type 1 and type 2 cytokine production is a complex process which involves the contribution of many factors (discussed in the next section entitled *Role of keratinocytes in the deviation of type 1/ type 2 immune responses*). It is clear that antigen presenting cells (APC) play a major role in the education of naïve T cells to develop into type 1 or type 2 cytokine-producing effector cells or, into the recently described, regulatory T cells. Antigen specific contact between APC and T cells via the T cell receptor (TCR) and concurrent triggering of co-stimulatory molecules are necessary events for this development (Figure 1). In addition to that, soluble factors produced by APC, but also many other different cell types, seem to contribute to the determination and preservation of the T-cell cytokine profile. This helps to maintain a sustained immune response as long as this response is needed. Finally, the immune response has to be switched off by regulatory mechanisms to avoid unnecessary damage to tissues. This is, at least partially, achieved by apoptosis of activated T cells. However, if (for unknown reasons) uncontrolled continuation of the mounted immune response takes place, the site of inflammation remains chronically activated as in the case of psoriasis. In connection with this, it is of interest to mention that cytotoxic and helper memory T cells can undergo proliferative renewal in the absence of MHC class I and class II molecules i.e. without the antigen presentation, respectively. In addition, some agents such as polyinosine:cytosine (poly I:C), lipopolysaccharides (LPS) and CpG oligodeoxinucleotides together with tissue factors which are found in the inflamed sites, such as IL-15, are found to stimulate the proliferation of bystander memory T cells in an antigen independent fashion. In case of psoriasis, it was shown that part of the T cells within the lesional skin is proliferating. Very recently, it was demonstrated in an animal model that local proliferation of T cells, without the influx of circulating T cells, is enough to initiate psoriatic lesions in nonlesional areas. The continuous presence of high numbers of activated T cells in the psoriatic lesional skin can result in perpetuation of this chronic inflammation.
Role of keratinocytes in the deviation of type 1 / type 2 immune responses.

Recently, significant progress has been made in the understanding of factors causing a type 1 cytokine-mediated immune response (Figure 2). For many years, it was thought that the major mediator of the type 1 T cell phenotype was IL-12, which is a heterodimeric cytokine formed by p40 and p35 subunits and is secreted mainly by dendritic cells and macrophages. It induces IFN-γ expression in naïve T cells and NK cells. This cytokine was also found to be secreted by other cells like neutrophils and keratinocytes, although the expression level was not high. IL-12 is expressed in high amounts in so called type 1 cytokine-mediated diseases which are characterized by an imbalance between type 1 and type 2 cytokines in favor of type 1 cytokines. Interestingly, studies showed that the p40 subunit of IL-12 was more important than the p35 subunit in the induction of autoimmune diseases, such as experimental autoimmune encephalitis. This finding gained a special meaning when it was discovered that p40 is a subunit of another cytokine named IL-23. In the biologically active heterodimer of IL-23, p40 is bound to a p19 molecule. Although IL-12 and IL-23 are undoubtedly similar in structure, they were found to have distinct roles in the development of type 1 immune responses. IL-23 was described, unlike IL-12, to have an effect on the proliferation and cytokine secretion of memory T cells. IL-23 can induce the expression of proinflammatory cytokine IL-17 by memory T cells, while IL-12 cannot. Recently, another member of the so-called IL-12 family of cytokines was described which was named IL-27. IL-27 is also a heterodimeric cytokine formed by a p28 subunit, resembling the p40 subunit of IL-12 and IL-23 and Epstein-Barr virus-induced gene 3 (EB-I3), which resembles to p35 subunit. IL-27 synergizes with IL-12 to activate...
naïve T cells and has no effect on memory T cells. It seems to be the very initial cytokine at the development of type 1 T cells as its receptors are found in undifferentiated T cells before the appearance of receptors for other IFN-γ-inducer cytokines. In addition to the IL-12 cytokine family, other cytokines were also reported to be important in the induction of type 1 immune responses. IL-15 (having resemblance to IL-2) and IL-18 (resembling IL-1) were found to contribute to the development of type 1 T cells. They are both secreted by a wide variety of cells. Very recently, IL-21 was reported to synergize with IL-15 and IL-18 to enhance the expression of IFN-γ by T and NK cells as well. It is a cytokine which is mainly produced by activated T cells. Another research group suggested that IL-21 suppresses the development of type 1 immune response showing that the real contribution of this cytokine to the type 1/type 2 immune deviation should further be investigated.

As it is demonstrated in many studies, keratinocytes do not simply form a physical barrier between the organism and the environment. They have a major contribution to the immediate non-specific innate immune response and are functionally involved in specific immune response by communicating with other immunocompetent cells via the release of cytokines and by interaction of membrane molecules during cell-cell contact. It is interesting to note that keratinocytes can play a role in the balance between type 1 and type 2 cytokine responses as well (Figure 3). As type 1 cytokine expressing T cells are important for the development of chronic inflammation and keratinocyte proliferation in psoriatic lesional skin, cytokines secreted from keratinocytes can affect the development and maintenance of type 1 immune response in the inflamed skin. Expression of p35 and p40 subunits of IL-12 was demonstrated at mRNA level in keratinocytes. IL-12 p35 was found to be

Figure 2. Regulation of type 1 T cells (T) by cytokines. Recently, new cytokines are added to the list of “IFN-γ inducers”. In addition to the very well known IL-12, IL-15, IL-18, IL-23 and IL-27 contribute to the development and maintenance of IFN-γ expressing type 1 T cells.
constitutively expressed while p40 expression was inducible with allergens. In addition to that, IL-12 heterodimer was detected in the supernatants of activated keratinocytes. In another study, both subunits of IL-12 mRNA were found to be constitutively expressed. In psoriatic lesional biopsies, IL-12 p40 subunit was found to be expressed higher than in normal and nonlesional skin, while the expression of p35 subunit was equal in lesional, nonlesional and normal skin. Surprisingly, IL-12 p70 heterodimer was detected in situ mainly in the dermal cell infiltrate, but not in keratinocytes. Having in mind that the p40 subunit of IL-12 is shared by IL-12 and IL-23, it would be interesting to know whether keratinocytes are capable of expressing the p19 subunit of IL-23 and eventually bioactive IL-23. If this is the case, the expression of IL-12/IL-23 p40 subunit by keratinocytes gains another dimension as IL-23 was described to be important for the activation of type 1 memory T cells.

The first study describing the expression by IL-15 from keratinocytes was conducted to demonstrate that IL-15 was induced in the skin after ultraviolet (UV) B exposure. IL-15 was found to be induced after UVB exposure in the HLA-DR+ cell population from epidermal sheets (presumed to be keratinocytes) and in cultured keratinocytes. In contrast, another study showed that the constitutive IL-15 mRNA expression in human keratinocytes was downregulated by UVB exposure. However, in a third study it was claimed that the

**Figure 3.** Cytokines involved in the inflammatory process in psoriatic lesions. Many cytokines play a role in the inflammatory process in the psoriatic skin. The bombardment of cytokines on dendritic cells (DC), T cells (T) and keratinocytes (KC) result in the activation, migration, recruitment and proliferation of these cells. T cells appear to be central in this inflammatory process.
expression of IL-15 was not constitutive, because it can not be detected in fresh keratinocytes, but could be induced during culturing upon stimulation. An interesting finding was that IL-15 could inhibit the apoptosis of keratinocytes. Because the expression of IL-15 was found to be increased in psoriatic lesional keratinocytes, it was suggested that IL-15 might play a role in the increased epidermal thickness by inhibiting apoptosis of keratinocytes. IL-18 is another candidate which might be important in the maintenance of type 1 immune response in chronically inflamed skin as a potent inducer of IFN-γ in activated T cells. Human keratinocytes were found to express IL-18 at mRNA level and intracellularly at protein level. Its in situ expression is increased in psoriatic lesional epidermis. This finding was confirmed by other groups. Overall, it appears that keratinocytes are able to express several cytokines which might induce and/or maintain type 1 cytokine response in chronically inflamed skin (Figure 3). Further studies are needed to reveal the expression of newly-described IFN-γ-inducing cytokines in keratinocytes and their possible contribution to the development and maintenance of psoriatic lesions.

Cross-talk of keratinocytes with immunocompetent cells in the psoriatic skin.
Psoriatic keratinocytes express molecules which enable them to communicate with the immunocompetent cells. Keratinocytes in psoriatic lesional skin were found to express major histocompatibility complex class II molecule HLA-DR, intercellular adhesion molecule (ICAM)-1, costimulatory molecule CD40, chemokines IL-8/CXCL8, IFN-γ-induced protein of 10 kDa (IP-10/CXCL10), monokine induced by IFN-γ (Mig/CXCL9), monocyte chemoattractant protein-1 (MCP-1/CCL2), and regulated-on-activation, normal-T-cell-expressed and -secreted (RANTES/CCL5), macrophage inflammatory protein-3a (MIP-3α/CCL20), E-cadherin, psoriasin and CDw60 which enable them to attract and activate T cells in the lesional skin that express relevant ligands / receptors lymphocyte function-associated antigen-1 (LFA-1), CD40L, CXCR1, CXCR3, CCR2, CCR4, CCR6 and integrin αEβ7. Many, if not all of the inflammation-related molecules expressed by keratinocytes were found to be induced by IFN-γ and TNF-α indicating that these cytokines, mostly produced by T cells, play a major role in the activation of keratinocytes. Supernatants of lesional, but not normal keratinocytes, induced the activation of purified CD4+ T cells emphasizing that keratinocytes are capable of producing soluble factors to activate T cells and to propagate the skin inflammation. Vice versa,
supernatants of T cell clones from lesional skin cause increased proliferation of nonlesional keratinocytes. Altogether, these studies reveal that there is a significant cross-talk between keratinocytes and T cells in the inflammatory reaction within the psoriatic skin.

**Disturbance of epidermal cell homeostasis in psoriatic skin.** The most striking histological change of psoriasis lesions is the epidermal thickening. Because of this, psoriasis was originally thought to be caused by a major defect in keratinocytes. It was demonstrated that psoriatic keratinocytes had an increased capacity to proliferate. The maturation cycle of psoriatic keratinocytes is approximately 10-fold faster as compared to that of normal keratinocytes. Abnormally proliferating keratinocytes in psoriatic skin were identified as stem cells which entered six- to seven-fold more into the S-G2+M phase of the cell cycle compared to the normal keratinocyte stem cells. The enhanced proliferation was explained by the increased expression of growth factors for keratinocytes such as transforming growth factor (TGF)-α, amphiregulin, keratinocyte growth factor (KGF), epidermal growth factor (EGF) and insulin-like growth factor (IGF)-I and increased expression of receptors for these growth factors such as IGF-I receptor and KGF receptor in the lesional skin as compared to normal skin. Not only growth factors, but also cytokines were found to contribute to the abnormal proliferative response of keratinocytes in lesional skin. IL-6 was described to be expressed in lesional skin and to cause hyperproliferation of keratinocytes. IFN-γ, which is known by its antiproliferative effects on normal keratinocytes in vitro, was found to cause less growth inhibition in keratinocytes from the lesional skin showing that these keratinocytes have an intrinsic proliferative defect as well. In one study, IFN-γ was reported to increase keratinocyte proliferation when it is injected intradermally into normal human skin. Another study revealed that IFN-γ-stimulated psoriatic keratinocytes show a decreased signalling via transcription factors interferon regulatory factor-1 (IRF-1) and signal transducer and activator of transcription (STAT)-1α, as compared to normal keratinocytes which suggests a fundamental defect in the growth and differentiation control of psoriatic keratinocytes. Similarly, an intrinsic defect of the calcium metabolism of psoriatic keratinocytes was determined supporting the view that keratinocytes are altered in psoriatic skin. Basement membrane alterations such as gaps, folding, and reduplication of the epidermo-dermal basement membrane were described as a result of epidermal overexpression of matrix metalloproteinase (MMP)-2 and MMP-19 as well.

Keratinocytes from lesional skin show several more phenotypical alterations. Keratin types
K6, K16 and K17 which are expressed in hyperproliferative conditions of the skin such as wound healing, but not in normal skin, are highly present in psoriatic lesional skin \(^{112}\). However, this aberration is probably not a primary defect, but rather caused by soluble factors in the inflamed lesional skin. Normal keratinocytes can express these markers as well upon in vitro stimulation with growth factors as demonstrated by the induction of K17 on keratinocytes after IFN-\(\gamma\) and IL-1\(\beta\) stimulation \(^{113}\). In addition to their increased capacity of proliferation, keratinocytes seem to be more resistant to apoptosis as compared to normal keratinocytes \(^{114}\). The expression of cathepsin D and zinc-alpha(2)-glycoprotein, two catalytic enzymes associated with apoptosis and desquamation, were upregulated in normal keratinocytes, but not in psoriatic keratinocytes, after IFN-\(\gamma\) stimulation \(^{115}\). Aberrant expression of differentiation markers were recognized in lesional keratinocytes. Involucrin and filaggrin, epidermal proteins playing a role in differentiation and maturation, are abnormally distributed in the psoriatic skin as compared to normal skin \(^{116}\). Transglutaminase which contributes to the terminal differentiation of keratinocytes and the formation of the cornified envelope was found to be expressed higher in psoriatic skin as compared to normal skin \(^{117}\). Another protein which is found to be expressed higher in lesional epidermis than in nonlesional and normal skin is the aforementioned Cdsn \(^{18}\). It is functional in the adhesion of terminally differentiated keratinocytes and therefore can play a role in the epidermal changes in psoriatic skin.

**Polymorphonuclear leukocytes in psoriatic lesions.** The presence of polymorphonuclear leukocytes is a striking histological feature of psoriasis lesions. These cells infiltrate the dermis and they collect in the epidermis to form Munroe’s microabscesses. However, in the dermal infiltrate of advanced lesions only a single or a few polymorphonuclear leukocytes could be seen \(^{118}\). Initial studies in the late 1970s indicated that complement cleavage products, e.g. C3a, in the scales were found responsible for the chemotaxis of these cells to the lesional skin \(^{119}\). In the 1980s, peritoneal dialysis was suggested as an effective therapy for extensive psoriasis in order to decrease the number and activity of neutrophils in the peripheral blood \(^{120}\). In addition, methotrexate, an effective therapy for psoriasis also appeared to inhibit the chemotaxis of neutrophils \(^{121}\). Further, in vitro studies to explain the polymorphonuclear cell infiltration to the lesional skin revealed that the activated mononuclear cells could produce chemoattractive factors for neutrophils \(^{122}\). In addition to that, newly formed lesions are found to be sequentially invaded by mononuclear cells followed by neutrophils \(^{123}\). Increased IL-8 receptor (CXCR1/CXCR2) expression was also
determined in neutrophils from psoriatic patients as compared to the normal controls, which can explain their increased migration into the lesional skin. Later on, psoriasin was described as a chemotactic factor for neutrophils in the lesional skin. Some authors suggested that neutrophils could be important in the island-like, "acute" inflammatory changes seen within the chronic inflammatory changes in the lesions mediated by T cells. The presence of neutrophils seems to be important for the perpetuation of skin lesions as agranulocytosis has been reported to result in the remission of psoriasis. The contribution of neutrophils to the hyperproliferation of keratinocytes was supported by the studies showing that human leukocyte elastase could cause in vitro and in vivo keratinocyte hyperproliferation. Acute generalized exanthematous pustulosis, another disease characterized by acute, extensive formation of nonfollicular sterile pustules on an erythematous background was found to be mediated by T cells. This suggests that neutrophils in the cutaneous inflammatory processes could be orchestrated by T cells by their IL-8 expression.

**Innate immune response participates in psoriatic inflammatory response.** Recent studies showed that the innate immunity might contribute to the pathological changes in psoriatic skin. The immune system can conduct an immediate aspecific reaction against encountered antigens which is called the innate immune response. This response is very important for the protection of the organism at the places where frequently many antigens are met, such as the respiratory tract, urinary tract, gastrointestinal tract and skin. Recent studies showed that antimicrobial peptides, which can be synthesized in high amounts in polymorphonuclear leukocytes, can also be produced by keratinocytes after an antigenic challenge. Studies showed that the functioning of keratinocytes in innate immunity is altered in psoriatic lesions. Two major classes of these peptides are b-defensins and cathelicidins, which have antimicrobial activities against bacterial, viral, and fungal pathogens. Keratinocytes were found to express human b-defensin (HBD)-1, HBD-2, HBD-3 and cathelicidin LL-37. These peptides were shown to be important for the defense of the skin against bacteria. HBD-2, HBD-3 and cathelicidin are expressed in high quantities in psoriatic skin lesions. Various defensins were described to have chemotactic activity for dendritic cells and T cells as well. For example, HBD-2 can bind to CCR6 which causes chemotaxis of immature dendritic cells and lymphocytes. By this way, defensins also show activity in the development of adaptive immune responses. High expression of defensins is suggested to take part in the inflammatory process in psoriatic skin. There are other innate immunity factors such as IL-8 and induced nitric oxide synthase (iNOS) which can locally be produced.
early in the inflammatory site. IL-8 is chemoattractant for neutrophils which contribute to the first line defense by phagocytosis of the bacteria. iNOS causes production of nitric oxide that is toxic for microorganisms. Both were found to be expressed in psoriatic skin in higher amounts compared to the normal skin. Enhanced expression of IL-8 and psoriasin in psoriatic skin can explain the influx of neutrophils into psoriatic skin. In addition to that, cytokines such as IL-1 and TNF-α can be produced in high amounts by local and rapidly invading cells to activate immediate immune response. These cytokines were demonstrated to be expressed in psoriatic skin as well. A group of nonclonal pattern recognition receptors, which recognizes molecular patterns shared by many pathogens, are important in the early recognition of microbial structures. One group of these receptors is represented by Toll-like receptors (TLRs). They signal via transcription factor NF-κB which is known to activate the promoter regions of numerous inflammatory genes. Recently, TLR1, TLR2 and TLR5 were shown to be expressed by keratinocytes. TLR1 and TLR2 were found to be highly expressed in the upper epidermis in lesional skin, while the expression of TLR5 in lesional epidermis was lower than that in normal epidermis. The expression of these TLRs was different in localization in psoriatic epidermis as compared to normal epidermis as well. In another study, TLR1 expression was found to be higher in basal keratinocytes of the lesional skin as compared to the normal skin. Besides, in psoriatic skin, the expression of heat shock proteins (HSPs) such as HSPs 27, 60, and 70 and their ligands such as CD91 is increased. These proteins and their ligands are able to induce an immune response by different ways: e.g. triggering NF-κB, stimulating dendritic cells and increasing IL-12 expression from dendritic cells.

In conclusion, it appears that many, if not all, factors of the innate immune response can participate in the inflammatory reaction in psoriatic skin. In addition, they can contribute to the functions which are related to the adaptive immunity such as activation of dendritic cells and T cells.

**Treatment of psoriasis**

In the nineteenth and the first half of the twentieth centuries, a commonly applied treatment of psoriasis was Fowler’s solution which was an arsenic mixture. Many other topical and systemic treatments for psoriasis, alone or in combination, were tried out through the years. In the 1920s, UV therapy was demonstrated to be effective in psoriasis which was followed by the development of Goeckerman (coal tar + UV) and Ingram (coal tar + UVB + anthralin) regimens to treat psoriasis. In the 1960s, the introduction of topical corticosteroids, especially
Table 1.

<table>
<thead>
<tr>
<th>Topical</th>
<th>Photo(chemo)therapy</th>
<th>Systemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids 141</td>
<td>Broadband UVB (290-320 nm)146</td>
<td>Methotrexate 150</td>
</tr>
<tr>
<td>Dithranol 142</td>
<td>Narrowband UVB (311 nm)147</td>
<td>Cyclosporine A 151</td>
</tr>
<tr>
<td>Tar 143</td>
<td>PUVA (320-400 nm)148</td>
<td>Acitretin 152</td>
</tr>
<tr>
<td>Tazarotene 144</td>
<td>Excimer laser (308 nm)149</td>
<td>Fumaric acid 153</td>
</tr>
<tr>
<td>Vitamin D analogues 145</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In occlusion, was a big breakthrough in the treatment of psoriasis until the side effects were recognized. In the early 1970s it was found that methotrexate, originally developed as an anticancer drug, could be used as therapy for psoriasis patients. In 1979, it was discovered that cyclosporine A was a good alternative for psoriasis therapy. An overview of currently applied (and widely approved) treatments of psoriasis is given in Table 1. Dependent on the severity of the disease, they are applied as a single therapy or in different combinations.

The common pitfall of all these classical therapies of psoriasis is their broad action-spectrum with a rather low selectivity. Therefore, these therapies are doomed to have many serious side effects such as nephrotoxicity caused by cyclosporine A and hepatotoxicity as a result of methotrexate therapy. Recent development in the treatment of psoriasis with new "biologics" seems to be promising as they are selective, effective and rather safe. Biologics are designed according to naturally occurring molecules that either have antagonistic effects on the inflammatory process or interfere with inflammation-promoting molecules. Examples for biologics are cytokines, cytokine inhibitors or antibodies against crucial molecules (membrane bound or in solution)154.

In the studies in this thesis methotrexate, cyclosporine A and UVB were used as therapy. For this reason only these treatments and their immunological effects are discussed in more detail below. Special attention is given to the UVB section as it was the major therapy studied in this thesis.

**Methotrexate**

Methotrexate is one of the oldest systemic therapies used in psoriasis 155. It is worldwide applied orally or intramuscularly in the treatment of moderate to severe psoriasis vulgaris. The low costs and the ease of use (once weekly application) made methotrexate a popular option in the therapy of this chronic disease. In a very recent comparative study, methotrexate was found to have a similar efficacy as cyclosporine A 156. However, the hepatic toxicity and the potential risk of myelosuppression caused by methotrexate limit its extensive use despite of its efficacy.
Methotrexate is a folic acid antagonist which suppresses cell proliferation. Originally, it was developed to treat cancer, but later it was extensively used in the treatment of rheumatoid arthritis as well. Its major therapeutic mechanism in psoriasis is believed to be the suppression of hyperproliferation of psoriatic keratinocytes. However, methotrexate appeared to have immunoregulatory effects as well. In vitro studies revealed that methotrexate killed proliferating T cells while not being effective on epidermal cells using comparable doses as in the clinical application for psoriasis. The selective deletion of activated T cells was found to be mediated by a CD95-independent apoptotic pathway. The chemotaxis of polymorphonuclear cells is inhibited by methotrexate in psoriasis patients. In addition, methotrexate was found to inhibit the functional activity of IL-1. In an experimental animal model of systemic lupus erythematosus, methotrexate decreased the leukocyte counts, immune complex formation and decreased the expression of IL-2, IL-4, IL-6 and IFN-γ. On the other hand, intracellular IFN-γ expression by peripheral T cells did not change after the methotrexate therapy of patients with rheumatoid arthritis. In another experimental mouse model of arthritis, methotrexate treatment resulted in decreased TNF expression while the expression of IFN-γ and IL-4 did not change significantly in serum and splenic cells. Similar results were found in patients with rheumatoid arthritis when peripheral T cells were examined for their cytokine expression before and after treatment with methotrexate. The cytokine expression profile of in vitro stimulated T cells from rheumatoid arthritis patients and normal controls changed from a type 1 cytokine to type 2 cytokine pattern after addition of methotrexate to the cell cultures. From all these data, it seems to be legitimate to think that the immunomodulation caused by methotrexate may be an important therapeutic mechanism causing improvement of psoriatic lesions.

**Cyclosporine A**

To date, cyclosporine A is used more than two decades in the treatment of psoriasis. Because of its suppressive effect on helper T cells, it was originally applied to prevent the rejection of organ transplantation. It passively enters into the cell and binds to cyclophilin causing inactivation of calcium dependent activation of the cell. Side effects such as nephrotoxicity and predisposition to malignancies are the limiting factors for its use. Not only T cells, but also Langerhans cells were found to have a dose-dependent reversible loss of function upon treatment with cyclosporine A. It was demonstrated that therapy of psoriasis with cyclosporine A resulted in a decrease in the number of Langerhans cells and T cells supporting the role of activated T cells in the pathogenesis of psoriasis. As
compared to the T cells before therapy, the peripheral blood T cells were not found to have a different phenotype or function after cyclosporine A therapy showing that there was no systemic alteration in the T cells. No alteration was found in the expression of adhesion molecules on keratinocytes and blood vessels after therapy with cyclosporine A. Keratinocytes continued to express higher levels of IL-6, TGF-β and K16 in the psoriatic skin after the therapy with cyclosporine A. These findings may explain, at least partially, the relapses which occur relatively quickly after the therapy. Although no apparent effect on the phenotype could be observed, cyclosporine A has been described to have a direct antiproliferative effect on keratinocytes.

**UVB**

**UVB therapy of psoriasis.** UVB therapy plays an important role in the long-term management of psoriasis. PUVA therapy of psoriasis, that is comprised of oral or topical application of photosensitizing psoralens followed by exposure to UVA (320-400 nm), was found to be superior to broad band (BB)-UVB (280-320 nm) therapy in efficacy. However, the development of narrow band (NB)-UVB therapy (311 nm) was an important step forward in the improvement of conventional BB-UVB therapy. Lamps emitting wavelengths lower than 300 nm are known to cause more erythema and discomfort of the skin, while having almost no antipsoriatic effect. The efficacy of NB-UVB therapy is comparable to that of PUVA therapy. In addition, NB-UVB therapy is cheaper (no use of psoralens) and easier to apply as it does not require eye protection due to the photosensitivity caused by psoralens. Therapy with NB-UVB-radiation seems to be less carcinogenic as compared to PUVA therapy. It can be used during pregnancy and childhood for patients with moderate to severe psoriasis. In practice, NB-UVB is applied 3 times weekly as this regimen was found to show the most efficacy with the least number of exposures.

NB-UVB dosage should be approximately 8 to 10 times higher than that of BB-UVB to reach the same erythemal effect. This means that the NB-UVB radiation is much less erythemogenic than the BB-UVB radiation. When NB-UVB and BB-UVB are applied at similar minimal erythema doses (MED), they seem to cause comparable immunological changes in the number of inflammatory cells in the irradiated skin and the expression of apoptosis markers and p53. In psoriasis patients, the clinical and histological healing of psoriasis lesions was found to be better after therapy with NB-UVB as compared to BB-UVB therapy.
Exposure to UVB has different facets. While it is very beneficial in the treatment of psoriasis patients, its carcinogenic character also needs great attention as skin cancers are known to be related to cumulative UV exposure and reported to be increased in prevalence. Many studies were performed to reveal these two opposite facets of UVB exposure in diseased and healthy skin. The role of UVB in carcinogenesis is beyond the scope of this thesis. The immunological alterations caused by UVB exposure in normal and psoriatic skin are separately discussed in the following sections.

**UVB-induced immunosuppression in normal human skin.** UV-induced skin tumors are very antigenic and easily rejected when they are transplanted to a naive syngeneic mouse. However, when the recipient mouse is exposed to UV-radiation prior to the transplantation, the grafted skin tumor is not rejected. This classical experiment at the end of the 1970s was the first proof that UVB can cause systemic immunosuppression. Contact hypersensitivity reactions induced by common sensitizers were also found to be suppressed by exposure to UVB in mice as well as in man. Interestingly, those people who have a history of skin cancer are more susceptible to the suppression of contact hypersensitivity reaction by UVB. This suggests that the immunosuppressive effect of UVB could be an important factor in the formation of UVB-induced skin cancer. Immunosuppression after UVB exposure can be explained as a physiological response to prevent an unnecessary immune reaction to UVB-induced neoantigens formed in the skin. This natural protection mechanism of the skin may cause serious problems at long-term, especially for people with a lighter skin complexion or a higher genetic susceptibility to UVB-induced immunosuppression.

UVB radiation influences the phenotype, function, distribution, and number of many different cell types in the human skin (Figure 4). In addition to changes in the resident cells of the skin, many immunocompetent cells from the peripheral blood are attracted into the skin upon UVB exposure. Langerhans cells, dendritic cells residing in the epidermis of normal skin, are able to take up antigens (also called haptens) in the skin and to migrate to the skin-draining lymph nodes to present haptens to T cells in order to mount a specific immune response. Different studies revealed that they are one of the major targets of the UVB radiation in the skin. Suppression of delayed type hypersensitivity reactions in man were found to be associated with the depletion of Langerhans cells in the irradiated skin. Exposure of Langerhans cells to UVB radiation resulted in a transient inhibition of antigen processing and presenting capacity of these cells showing that functional changes
in Langerhans cells upon UVB exposure can be expected in vivo as well\textsuperscript{183-185}. The recovery of Langerhans cell functions after the UVB exposure was found to be poorer than that after the UVA exposure suggesting that UVB-induced immunosuppression could be more sustained \textsuperscript{186}. The functional expression of costimulatory molecules on the Langerhans cells were also found to be disturbed by UVB exposure which could seriously impair the Langerhans cell-T cell interaction \textsuperscript{187-189}. Low dose UVB-exposed Langerhans cells preferentially induce type 2 T cell activation, instead of type 1, which might be explained by the alteration in the expression of costimulatory molecules after the irradiation \textsuperscript{190}. The fate of Langerhans cells after UVB exposure is still a point of discussion. Human Langerhans cells were found to undergo apoptosis after UVB exposure in vitro \textsuperscript{188}. On the other hand, they are still able to migrate to afferent lymph nodes after UVB irradiation in vivo \textsuperscript{191}. A recent study revealed that exposure to 6 MED UVB in vivo causes apoptosis of very few Langerhans cells while most of them emigrate from the skin \textsuperscript{192}. The migration of Langerhans cells after UVB exposure could be explained by increased expression of IL-1 and TNF-a which are upregulated in keratinocytes after UVB exposure and are known to induce/enhance the Langerhans cell migration \textsuperscript{193-196}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{cell Trafficking.png}
\caption{Cell trafficking in high dose UVB-exposed human skin. A heavy traffic of immunologically competent cells takes place in the high dose (4 MED)UVB-exposed normal skin. Shortly after UVB irradiation, Langerhans cells (LC) either leave the skin to reach to afferent lymph nodes, or die. Resident epidermal T cells (T) die by apoptosis as well. The influx of neutrophils (N) and macrophages (M) becomes prominent first in dermis and then in epidermis. CD4+ T cells invade the dermis and, later on, epidermis as well which stay somewhat longer than neutrophils and macrophages in the irradiated skin. A number of cytokines dominate the micromilieu of the skin after the UVB exposure which contributes to the immunosuppressive changes. These cytokines were reported to be mainly secreted by keratinocytes, macrophages and T cells. Yet, it is not clear whether the influx of neutrophils has any significance in the UVB-exposed skin.}
\end{figure}
Langerhans cell depletion in UVB-exposed skin is accompanied by an influx and local proliferation of CD11b+HLA-DR+ cells in the dermis and epidermis. These cells were defined as UV-macrophages with a peculiar characteristic of high IL-10 and low IL-12 expression. These cells could be important in the immunosuppressive function of UVB radiation as the inhibition of CD11b or its ligand iC3b reversed the immune tolerance which is induced by UVB exposure.

After UVB exposure, neutrophils are the first cells invading the skin. Proinflammatory cytokines such as IL-8 and TNF-α, were suggested to play a role in the accumulation of these cells in the irradiated skin. The significance of the influx of neutrophils to the UVB-irradiated skin has not yet been studied in detail.

In addition to the influx of neutrophils and macrophages into the UVB-exposed skin, T cell homeostasis was found to be altered in the irradiated skin. T cells are present in low numbers in both the dermal and epidermal compartments of unirradiated normal skin. The epidermal compartment contains 2% of the total T cell population in normal skin and most of these cells are CD8+ memory T cells. Most dermal T cells are CD4+ memory T cells. These T cells are assumed to be important in the immunosurveillance of the skin. Supporting this concept, renal transplant patients, who are known to be prone to skin infections and cancers, were found to have a decreased number of cutaneous T cells.

After a single exposure to an erythemal dose of solar-simulated or UVB radiation there is a clear change in the composition of the T cell population in normal skin. Intraepidermal T cells disappear within 48 h after the exposure while there is an influx of T cells into the dermis after 24 h following the irradiation. After 1 week, the epidermis is repopulated by CD4+ memory T cells lacking activation markers. Although there is no in situ evidence, in vitro studies revealed that apoptosis of intraepidermal T cells could be the major mechanism in the depletion of these cells from the normal human epidermis after the UVB exposure. The influx of T cells into the skin after UVB exposure is caused by a modulation in the expression of adhesion molecules on endothelial cells and keratinocytes.

The significance of the changes in the cutaneous T cell population after exposure to UVB is not clear. As it was mentioned earlier in this section, UVB-irradiated dendritic cells display an altered accessory signaling for T cells and can only activate the proliferation of type 2 T cells. In line with this data, it was shown by di Nuzzo et al that the in vivo exposure of human skin to UVB results in the development of type 2 T cells with high expression of IL-4. As type 2 T cells are known to counteract the effects of type 1 T cells and downregulate the development of cellular immune response, these findings can, at
least partly, explain the immunomodulation which is seen in the UVB-irradiated skin. In mouse experiments, it was shown that the UVB-induced suppression of contact hypersensitivity is induced by antigen specific suppressor T cells supporting the concept of the development of a new population of T cells in the irradiated skin \textsuperscript{211,212}. Suppressor T cells found in the UVB-irradiated skin were thought to be type 2 T cells because of their IL-10 production\textsuperscript{213}. However, these T cells could also represent the recently defined regulatory T cells which express IL-10 without expressing other classical type 2 cytokines such as IL-4. Naturally occurring CD4\textsuperscript{+}CD25\textsuperscript{+} regulatory T cells, which suppress the activation of CD4\textsuperscript{+} T cells by cell contact, and induced regulatory T cells (namely Th3 and Tr1), which mediate the T cell suppression by the expression of TGF-\(\beta\) and IL-10, have been described \textsuperscript{214}. Supporting the concept of development of regulatory T cells in the UVB-exposed skin, it was recently published that UVB-exposed dendritic cells induced the development of nonproliferating T cells which express high amounts of TGF-\(\beta\) \textsuperscript{215}. In addition, the T cells present in the UVB-irradiated skin of mice after dinitrofluorobenzene sensitization expressed CTLA-4 and produced high levels of IL-10, TGF-\(\beta\), and IFN-\(\gamma\); low levels of IL-2; and no IL-4 resembling the Tr1 cells. Inhibition of the CTLA-4 expression resulted in the reversal of the tolerance to the contact sensitizer \textsuperscript{216}. These results suggest that, in addition to type 2 T cells, regulatory T cells are operational in the suppression of immune responses in the UVB-exposed skin. The UVB-induced changes in the cellular composition of the skin are mediated by the cascade of UVB-induced molecular changes.

**UVB-induced molecular changes.** Trans-urocanic acid is a common constituent of the epidermis and is transformed into cis-urocanic acid upon absorbing UVB-radiation. Another UVB absorbing component in the skin is DNA within the cells. Cyclobutane pyrimidine dimers represent UVB-induced damage of the DNA. It was reported that both cis-urocanic acid and cyclobutane pyrimidine dimers play a role in the development of UVB-induced immunosuppression\textsuperscript{217-219}. Inevitably, there are numerous other local changes in the UVB-irradiated skin in addition to the aforementioned altered resident skin cells and the different cell types that infiltrate the irradiated skin. Detailed description of these factors is beyond the scope of this thesis.

**Immunological effects of UVB therapy on the psoriatic skin.** In an earlier study on the possible therapeutic effects of UVB-radiation on psoriatic skin, it was suggested that this therapy might decrease the influx of neutrophils into the lesional skin by the suppression
of chemoattractant leukotriene B4. In addition, the functional capacity of neutrophils, such as chemokinesis and phagocytosis, was found to be decreased in the psoriatic patients after UVB therapy. The soluble factor IL-2 and T cell activity were found to be decreased in the peripheral blood of psoriasis patients treated with the UVB therapy. A significant decrease in the IL-6 and TNF-α expression in the suction blister fluids from the psoriatic lesional skin and serum was detected after UVB therapy. Keratinocytes seemed to be directly or indirectly affected by UVB exposure. The expression of KGF and KGF receptor in psoriatic lesions was found to be suppressed after the therapy with UVB-radiation. Once appreciated the role of T cells in the formation of psoriatic skin lesions, the possible effects of different therapies on T cells were investigated. Krueger et al reported that 90% of the epidermal T cells were depleted after UVB treatment of psoriasis lesions which accompanied the reversal of the keratinocyte pathology, while most of the dermal T cells remained in the skin. This depletion, which was suggested to be due to the apoptotic cell death, was more prominent after therapy with NB-UVB. Alternatively, reduced expression of adhesion molecules by cutaneous endothelial cells (necessary for T cell diapedesis) and decreased expression of skin homing molecules on T cells were also proposed as therapeutic mechanism.

**Aims of the thesis**

Support for the role of type 1 T cells in the pathogenesis of psoriasis is well documented. The improvement caused by anti-psoriasis therapy could be mediated by an altered cytokine expression in these T cells within the psoriatic lesions. The aim of this study was to reveal possible changes in the cytokine expression in the lesional skin of psoriasis patients after systemic therapies. Because UVB irradiation is known to have immunosuppressive effects that are, at least partially, mediated by altered cytokine expression in normal skin, the studies in this thesis are in large part focused on UVB therapy. To investigate the changes in cytokine expression by cutaneous T cells following UVB exposure in psoriatic skin, we first applied a local single 4 MED BB-UVB to lesions of psoriasis patients. Before, two days and 14 days after the exposure, we obtained skin biopsies and determined T cell numbers and the expression of IFN-γ and IL-4 in situ; intracellular IFN-γ and IL-4 expression from stimulated dermal T cells in vitro; and the expression of the mentioned cytokines at mRNA level in extracts from skin biopsies (chapter 2). To see whether the therapeutical use of UVB radiation on psoriasis lesions affects type 1/ type 2 cytokine balance, we treated psoriasis patients with low dose repetitive NB-UVB radiation (regular UVB therapy) and
determined T cell numbers and cytokine expression in lesional skin biopsies using similar techniques as in our previous study. In addition to the expression of IFN-γ and IL-4 we also determined the levels of immunosuppressive cytokines IL-10 and TGF-β produced by in vitro stimulated lesional dermal T cells (chapter 3). In chapter 4, we focussed on the UVB-induced expression of IL-4 by neutrophils and the possible role these cells play in the altered cytokine expression by cutaneous T cells. Because of the possible differences in the normal and psoriatic skin, UVB-induced expression of IL-4 by neutrophils in the psoriatic skin was investigated in a separate study (chapter 5). IL-10 is known to be expressed in UVB exposed skin by CD11b+HLA-DR+ cells which generally have been referred as UV-macrophages 197,198. However, as these markers are also expressed by neutrophils and these cells are known to infiltrate the UVB-exposed skin as well, we examined in chapter 6 whether neutrophils could contribute to the IL-10 expression in the irradiated-skin. Several cytokines are defined to be important in the induction of IFN-γ expression by T cells 230. Recently, IL-23 was discovered and found to be important in the stimulation of IFN-γ expression in memory T cells 63. In chapter 7, we investigated the expression of IL-23 in normal and psoriatic skin and demonstrated that keratinocytes are able to express this cytokine. In the next chapter (chapter 8), we studied the expression of IL-12, IL-15, IL-18 and IL-23 in the psoriatic lesional skin before and after the NB-UVB therapy and the correlation of the changes in the expression of these cytokines with the expression of IFN-γ which take place in the inflammatory reaction in the lesional skin. In chapter 9, we determined the effects of methotrexate and cyclosporine A on T cell numbers and the expression of IFN-γ and IL-4 in psoriatic lesional skin by immunohistochemistry.
References


70. Wurster AL, Rodgers VL, Satoskar AR et al. Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into interferon gamma-producing Th1 cells. *J.Exp.Med.* 2002; 196: 969-77.


Wei L, Debets R, Hegmans JJ et al. IL-1 beta and IFN-gamma induce the regenerative epidermal phenotype of psoriasis in the transwell skin organ culture system. IFN-gamma up-regulates the expression of keratin 17 and keratinocyte transglutaminase via endogenous IL-1 production. *J.Pathol.* 1999; 187: 358-64.


