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Perspective

New Dermatological Agents for the Treatment of Psoriasis

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Introduction

Psoriasis is a complex inflammatory skin disease in which the epidermis becomes markedly hyperplastic and infiltrated with cytotoxic T-cells. Several well-characterized systemic therapies are available such as methotrexate, retinoids, and cyclosporine, but limitations on safety prevent their widespread application. Topical or external therapies, in addition to ultraviolet (UV) light, include glucocorticoids, analogues of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), and the retinoid tazarotene. Remarkably, each of these treatment modalities appears to be directed toward very different molecular targets. This review will summarize our knowledge of the mechanisms of established therapies with emphasis on possible common elements and will also explore therapeutic approaches still in the experimental stage.

About 2% of the adult population is affected by psoriasis, and its incidence increases with age.[1] Two major components of the disease have been recognized since the late 1800s. The first is a striking accumulation of superficial epidermal scale, a greatly increased hyperplasia and thickening of the living epidermis, and a changed pattern of histological differentiation within the epidermis itself. The second is an exudate or outpouring of inflammatory cells into the dermis and an enlargement of dermal blood vessels, both of which contribute to the erythema, induration (or hardening), and raised appearance of the psoriatic plaque.[2,3] The effectiveness of newer, highly specific immunosuppressive therapies indicates that T-cell-driven inflammation is critical for the pathogenesis of the disease,[3,4] and model studies show that T-cell infiltration as well as specific cytokines released from the activated T-cells can induce hyperproliferation of the epidermal keratinocytes.[5,6] The mechanisms of long-standing therapies such as UV light and oral methotrexate, initially assumed to act by inhibiting epidermal proliferation, are being reevaluated. Both agents also clearly induce T-cell apoptosis, and it appears possible that a major therapeutic effect of ultraviolet B (UVB) light is to eliminate epidermal cytotoxic T-cells.[7−9] Ongoing studies are having a significant impact on the understanding of the basic pathophysiology of psoriasis; through this work, additional therapeutic targets for psoriasis may be identified at the molecular level.

Pathophysiology of Psoriasis. Epidermal hyperproliferation and scaling in the psoriatic plaque is the result of a 40-fold increase in the number of epidermal mitotic cells and a reduction in the transit time through the living layer from about 12 to 2 days.[10] There are also widespread changes in epidermal histology and gene expression. The nonliving cornified or outermost layer of skin becomes much thicker and disorganized.[6,11] The final living layer of the epidermis, the granular layer, is no longer recognizable at the histological level, and at the same time, certain proteins identified with the granular layer, such as filaggrin, are suppressed.[11] In addition, the major cell type of the epidermis, the keratinocyte, expresses a number of new structural proteins, including the intermediate filament subunits

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keratins 6 and 16 (K6 and K16) as well as additional precursor proteins for the epidermal cornified envelope.[12,13] Gene expression follows a pattern that is very similar to hyperproliferative epidermis during wound healing.[14,15]

A complex mixture of growth factors and cytokines is induced in psoriasis. Prominent among these are autocrine growth factors for the epidermal keratinocyte, such as transforming growth factor-α (TGF-α), amphiregulin, and heparin-binding EGF-like growth factor (HB-EGF), which bind the epidermal growth factor receptor (EGFR).[16,17] The keratinocyte in culture produces a number of proinflammatory cytokines in response to treatment with interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α,) and interferon-γ (IFN-γ), suggesting that keratinocytes may amplify the effect of infiltrating inflammatory cells.[18−20] In addition, keratinocyte expression of the leukocyte trafficking receptor, ICAM-1, is induced by IFN-γ[21]. T-cell-derived cytokines can induce keratinocyte mitogenesis, suggesting reciprocal interactions between the stationary and infiltrating cells. For example, injected granulocyte-macrophage colony-stimulating factor (GM-CSF) induces epidermal mitogenesis in human skin,[22] and a combination of cytokines from lesional T-cell supernatants, IFN-γ, GM-CSF, and IL-3, selectively induces mitosis of epidermal keratinocytes freshly derived from the uninvolved epidermis of psoriatic patients but not from normal individuals.[5]

The immune and inflammatory cell types found in psoriatic plaque are quite complex. Neutrophils accumulate in the stratum corneum, and T-cell and macrophage levels are elevated in both the epidermal and dermal layers of skin.[5,6,23] Immunocytes (T- and B-cells and monocytes) are also activated in the peripheral blood in proportion to the severity of the disease.[24] The predominant T-cell type in the epidermis is the cytotoxic CD8+ T-cell, whereas the major T-cell type in the dermis is the CD4+ helper T-cell.[23] Psoriasis therapy is correlated with clearance of CD8+ T-cells from the epidermis,[15] and treatments such as UV light that cause complete clearance also produce the longest remissions.[23,25] Inflammatory cytokine expression in the psoriatic plaque follows a type 1 pattern, characterized by high levels of IFN-γ and IL-2, consistent with a cell-mediated immune response,[26] and levels of the type 2 cytokines, IL-4 and IL-10, which promote the humoral immune response, are correspondingly quite low.[27] Preliminary clinical studies have found that subcutaneous injection of IL-10 or IL-11 in psoriasis patients induces an induction of type 2 or an inhibition of type 1 cytokine expression along with improvement in the disease. The cytokine environment of the plaque may therefore promote the cell-mediated T-cell responses that are critical for the pathogenesis of the disease.[4,28]

Inhibition of T-cell activation is also therapeutically effective in psoriasis. The immunosuppressive agents cyclosporine and FK506 block IL-2 expression which is required for T-cell growth and survival.[29] The fusion toxin, DAB_{389}IL-2 (IL-2 fused to diphtheria toxin), is an injectable protein designed to target primarily activated cytotoxic T-cells, which have a 1000-fold higher affinity for IL-2 than resting T-cells.[30] CTLA4-Ig (BMS-188667) blocks T-cell interactions with antigen-presenting cells and prevents T-cell activation through the T-cell receptor.[3] The beneficial effects of these therapies (FK506, DAB_{389}IL-2, and CTLA4-Ig are still considered experimental) suggest that the presence of activated T-cells is critical for development and maintenance of the psoriatic plaque. Although genetic susceptibility to psoriasis does not follow simple Mendelian inheritance, the elevated association of several HLA subtypes with the disease[31] also indicates that T-cell activation is involved. One of the most convincing models for psoriasis at the histological level involves injection of activated autologous T-cells into immunodeficient mice grafted with unaffected skin from psoriasis patients.[6,32] These grafts become markedly hyperplastic, vividly demonstrating the powerful impact that the activated T-cell can have in skin.

A striking aspect of psoriasis etiology is its limitation to discrete plaques, with a sharp boundary between involved and uninvolved areas of skin.[1] The appearance of the disease suggests that its spread is strongly inhibited by normal, nonhyperproliferative epidermis. Normal-appearing skin in a susceptible individual can often be converted to a psoriatic plaque by wounding or mild abrasion (the Koebner effect),[33] presumably because the cascade of events initiated by epidermal hyperproliferation is not as easily reversible as in normal individuals. Dysregulation of epidermal proliferation and differentiation in transgenic mice, either by overexpression of amphiregulin, a heparin-binding ligand for the EGF receptor,[16] or by aberrant, suprabasal expression of cell adhesion molecules normally found in the basal layer only, such as the integrin subunit β_1,[34] can produce a psoriasis-like phenotype. Thus, disruption of normal controls on epidermal homeostasis appears sufficient to induce T-cell infiltration and inflammation of the epidermis and may also be a critical step in disease pathogenesis.

Current Therapies and Their Mechanisms of Action. The range of therapeutic options for psoriasis is quite extensive and has been the subject of some excellent recent reviews.[1,35] The severity of psoriasis is related to the percentage of body surface area involved and therapy is designed accordingly. Psoriasis which is limited in extent can be treated topically. Topical therapies include coal tar and anthralin, which are poorly understood mechanistically. Ligands for several nuclear receptors of the steroid superfamily, including glucocorticoids, vitamin D_3 analogues such as calcipotriol, and the retinoid tazarotene, are also therapeutically effective by a topical route. For cases which involve large areas of the body (20% or greater), UV irradiation and/or systemic therapies are required. UV therapies include UVB light, which is most effective if limited to a narrow wavelength range near 312 nm,[9] and long-wavelength UVA light in the presence of a sensitizer such as psoralen (PUVA).[36] Systemic retinoids, methotrexate, fumaric acid esters,[37] and immunosuppressive agents such as cyclosporine are also effective orally.[1] Current monotherapies have significant drawbacks and are often used in combination. For example, UV in combination with systemic retinoids increases efficacy and reduces the UV dose and consequent long-term risk of epidermal neoplasia. Topical glucocorticoids, while extremely effective as local antiinflammatory agents,

induce skin thinning as a side effect; on the other hand, topical retinoids stimulate both epidermal hyperplasia and new dermal collagen synthesis.[38] Significantly, the combination of the topical retinoid tazarotene with a glucocorticoid is more effective than tazarotene alone.[39] Calcipotriol also works effectively as an adjunct to glucocorticoids and UV light.[35]

In the following sections, we discuss the properties, mechanisms of action, and possible future evolution of major therapeutic modalities for psoriasis. A subsequent section presents therapies under investigation. Finally, on the basis of our current knowledge of disease pathogenesis, we take a look at potential new drug targets.

Ligands for the Nuclear Hormone Receptors

Three major classes of psoriasis therapy, glucocorticoids, retinoids, and analogues of $1,25(OH)_2D_3$, all bind to and activate members of the steroid nuclear hormone receptor superfamily. This family of receptors has a conserved modular structure that includes both DNA and hormone-binding domains; furthermore, activation of these receptors by ligand can produce pleiotropic effects on mRNA expression, including both inhibition and induction of hormone-specific genes. However, because of systemic side effects, the use of these drugs in psoriasis, except for retinoids, is limited to topical application.

Glucocorticoids. Activation of the glucocorticoid receptor (GR) inhibits the activity of the proinflammatory enhancers NF-κB and AP-1, and this may account for the very potent antiinflammatory activity of glucorcorticoids.[40] Glucocorticoids have a rapid therapeutic effect in psoriasis, but duration of use is limited by tachyphylaxis (resistance to prolonged therapy) and skin atrophy or thinning. A posttreatment rebound of the disease is also well-documented.[25] Since topical glucocorticoids constitute a well-established and well-characterized treatment for psoriasis, they will not be discussed in this review.

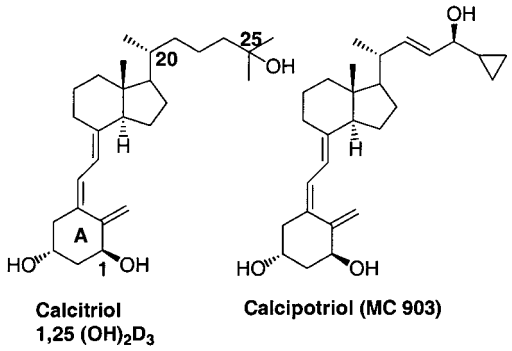
Vitamin D_3 Analogues. Calcitriol or $1,25(OH)_2D_3$ is the active form of vitamin D_3 which binds the nuclear vitamin D receptor (VDR).[41] Calcitriol and several of its analogues have been used topically, with limited side effects, to treat psoriasis. In addition to the classical target organs of intestine and bone, skin is also a target organ of VDR agonists. Cultured keratinocytes and dermal fibroblasts contain receptors for $1,25(OH)_2D_3$,[42] and $1,25(OH)_2D_3$ is a potent stimulator of epidermal cell differentiation[43,44] that inhibits the proliferation of keratinocytes.[45] The first indication that vitamin D_3 analogues might be beneficial in psoriasis was provided by the observation of clinical improvement in a patient during a study of oral 1α-hydroxyvitamin D_3 ($1\alpha(OH)$-D_3) in osteoporosis.[46] This finding has been supported by a number of additional studies of analogues of $1,25$-$(OH)_2D_3$. Oral vitamin D_3 therapies are severely complicated by hypercalcemia, however, and therefore it is very significant that topical application of the analogues has proven to be clinically effective.[47] The most widely used topical analogue of $1,25(OH)_2D_3$ is calcipotriol (MC 903). In a clinical study involving 167 chronic plaque psoriasis patients with twice daily application of calcipotriol ointment (50 μg/g ointment), complete clearing was observed in 26% of the patients and significant improvement was observed in 50−70% of the patients.[48] Approximately 20% of the patients reported cutaneous irritant reactions to topical calcipotriol in various clinical studies. Although calcipotriol is ~100-fold less potent than $1,25(OH)_2D_3$ as a hypercalcemic agent when tested in rodents,[49] hypercalcemia can be dose-limiting in topical therapy, and >100 g/week of calcipotriol ointment (50 μg/g) is contraindicated.[35] In addition to calcipotriol, new synthetic analogues such as tacalcitol, lexacalcitol (KH 1060), GS 1500, CB 1093, EB 1213, maxacalcitol (MC 1275 or OCT), and MC 1288 with potent cell differentiating and cell proliferation inhibitory effects but with reduced effects on calcium metabolism have been synthesized, mainly for topical use. Numerous clinical studies have demonstrated the efficacy of some of these analogues, including tacalcitol (4 μg/g) and maxacalcitol (25 μg/g).[50,51]

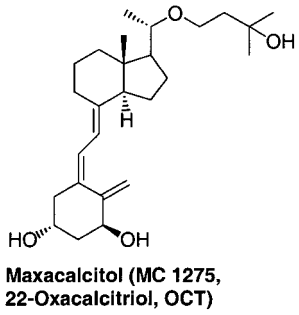
A. Mechanism of VDR Ligand Activity in Psoriasis. VDR agonists such as $1,25(OH)_2D_3$ and calcipotriol have been shown to induce terminal differentiation accompanied by inhibition of cell proliferation in murine and human keratinocyte cultures.[43,44,52] VDR agonists also induce cornified envelope formation and increased levels of the differentiation markers transglutaminase I (TGase I) and involucrin.[45] In psoriatic plaque treated with $1,25(OH)_2D_3$ or calcipotriol, the most rapid changes (within 2 weeks) appear to be a reduction in cell proliferation and in neutrophil content as measured by staining for elastase.[53,54] In addition, IL-10 levels in the plaque are elevated and IL-8 is reduced within 3 days of calcipotriol treatment, suggesting an early effect of calcipotriol on critical cytokine regulatory pathways.[55] Activation of B- and T-lymphocytes induces the expression of VDR, and $1,25(OH)_2D_3$ potently inhibits the proliferation of mitogen-activated T-lymphocytes.[56,57] In mouse models of autoimmune disease and transplant rejection,[49] $1,25(OH)_2D_3$ and its analogues have a modest immunosuppressive effect that may also contribute to efficacy in psoriasis.

B. Regulation of Gene Expression by VDR Agonists. Most of the biological actions of vitamin D_3 analogues are mediated by VDR, which is a ligand-dependent transcription factor belonging to the superfamily of steroid/thyroid hormone nuclear receptors.[58] $1,25(OH)_2D_3$ and its analogues modulate gene expression through a heterodimer between VDR and the retinoid X receptor (RXR).[41,59] VDR must heterodimerize with RXR for binding to the vitamin D_3 response elements (VDREs) present in the promoter region of responsive genes. The VDREs are direct repeats of 5′-AGGTCA-3′ motifs separated by four nucleotides (DR4).[59] Most $1,25(OH)_2D_3$-inducible genes, such as osteocalcin, osteopontin, and calbindin 9K, contain VDREs and are involved in calcium homeostasis and metabolism.[49,60] There are others, such as the cell cycle inhibitor p21, which also can induce keratinocyte differentiation, and this may be relevant to the antiproliferative activity of these compounds in psoriasis.[61,62] VDR agonists also inhibit the expression of some critical cytokines, including IL-2 and GM-CSF.[63] Negative regulation of GM-CSF appears to occur through an entirely different class of DNA binding site than a VDRE,[63] however, suggesting that the mechanisms for VDR-mediated inhibition of

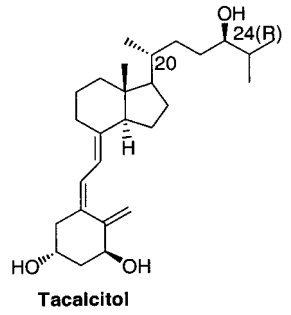
gene expression may be more diverse and complex than for induction.

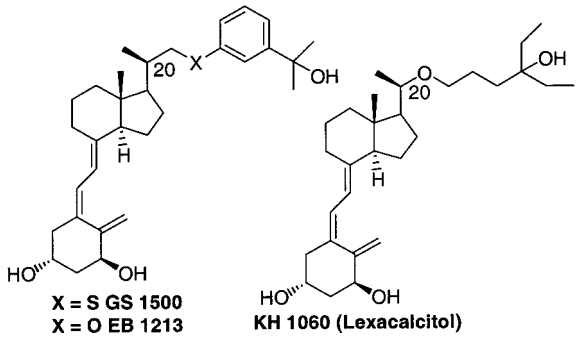
C. Structure−Activity Relationships Among VDR Ligands. Calcipotriol (MC 903, dovonex, calcipotriene, psorcutan) is the most widely used $1,25(OH)_2D_3$ analogue for the topical treatment of psoriasis.[64] It is a side chain-modified analogue of $1,25(OH)_2D_3$ with an *E*-double bond at C-22, a hydroxy group at C-24, and a cyclopropyl ring at C-25, designed to be metabolized rapidly in systemic circulation. Although calciptriol, as noted above, is 100 times less active than $1,25(OH)_2D_3$ in causing hypercalcemia, it is as potent as $1,25(OH)_2D_3$ in inducing terminal differentiation and inhibiting cell proliferation of cultured human keratinocytes.[65] Tacalcitol (curatoderm, TV-02 ointment) or 1α,24(*R*)-dihydroxyvitamin D_3 is another side chain-modified analogue with decreased hypercalcemic activity compared to $1,25(OH)_2D_3$[66] that has been shown to be effective in the topical treatment of psoriasis.[67]

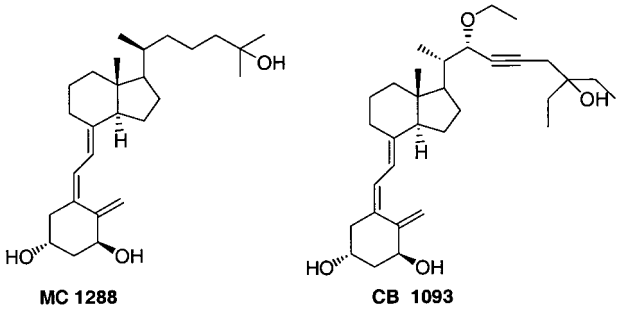




Substantial research has been carried out to probe the molecular basis for the design of therapeutically effective $1,25(OH)_2D_3$ analogues.[41,49] The overall effect of the analogues is a function of the interaction with four proteins: the nuclear receptor VDR, the serum vitamin D-binding protein (DBP), the vitamin D 24-hydroxylase, and a recently proposed membrane receptor. Binding to VDR appears to be required for therapeutic efficacy, and removal of the 1α-hydroxyl group in ring A sharply reduces binding affinity. The vitamin D side chain can be extensively modified, however, with little effect.[49] Interestingly, there can be a significant disparity between VDR binding and in vitro potency in tests of biological activities such as growth inhibition or bone resorption.[49,65,68,69] The C-20-*epi* analogues of $1,25(OH)_2D_3$ such as GS 1500, EB 1213, and KH 1060 are particularly striking in this regard. While GS 1500 (K_d = 0.089 nM) and EB 1213 (K_d = 0.073 nM) bind to VDR with comparable affinity to $1,25(OH)_2D_3$ (K_d = 0.065 nM) and calcipotriol (K_d = 0.31nM), the IC_{50} values of GS 1500 and EB 1213 in the growth inhibition of human keratinocytes were much lower (0.28 and 0.11 nM, respectively) than those of $1,25(OH)_2D_3$ (IC_{50} = 50 nM) and calcipotriol (IC_{50} = 32 nM).[65] The C-20-*epi* synthetic analogues GS 1500, EB 1213, and KH 1060 are also more potent than $1,25(OH)_2D_3$ in inducing gene expression from a VDRE transfected into human keratinocytes or rat osteosarcoma cells.[65,69] Recent evidence suggests that the 20-*epi* analogues are more potent inducers of gene transcription through VDR because they produce a conformational change that increases the half-life of VDR within the cell in comparison with nonepimerized analogues.[68] The 20-*epi* analogues also interact differently with the VDR ligand-binding domain, causing an altered pattern of VDR protease sensitivity.[69]







The structural elements that are necessary for DBP binding are quite different than those required for VDR binding. While the 1α-hydroxy group does not affect DBP binding, the side chain plays a crucial role. In general, the side chain-modified analogues of calcitriol have low affinity to serum DBP, leading to a more rapid clearance from circulation that limits their calcemic activity.[41] The vitamin D analogue maxacalcitol has a 500-fold lower affinity than $1,25(OH)_2D_3$ for serum DBP, and it is also rapidly cleared from circulation with a net reduced calcemic effect.[70]

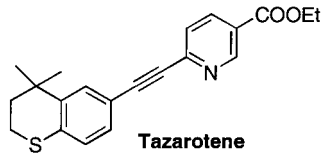
Oxidation at C-23 and C-24 by vitamin D 24-hydroxylase results in side chain cleavage and inactivation,[71,72] and this is the major metabolic degradation pathway for vitamin D analogues. The 24-hydroxylase products of certain analogues retain very significant biological activity, however, and intracellular retention of active metabolites has also been proposed to explain the greater potency of some 20-*epi* analogues, including KH 1060.[71] Vitamin D analogues may also undergo epimerization at the 3-position with retention of activity. Epimerization alters metabolism by 24-hydroxylase[72] and thus can affect analogue stability.

In addition to regulation of gene transcription, 1,25-$(OH)_2D_3$ is also implicated in rapid effects in target cells that occur too quickly to be explained by gene transcription.[73] The acute effects include elevation of intracellular calcium, activation of protein kinase C, and rapid induction of intestinal calcium uptake (transcaltachia).[74] A nonnuclear or plasma membrane pharmacophore has been proposed, but the identity of such an additional Vitamin D receptor is unclear.[41] The differential ability of analogues to elicit rapid effects such as transcaltachia could have an effect on their overall therapeutic activity.

Vitamin A Derivatives: Retinoids. Physiological oxidation of vitamin A (retinol) produces a pharmacologically active retinoid, *all-trans*-retinoic acid (RA), that is critically involved in embryonic development and in maintenance of normal differentiation in many epithelial tissues after birth.[75] Retinoids have now been shown to have a wide range of clinical applications in dermatology and cancer.[76] A total of six retinoic receptors have been identified: the retinoic acid receptors (RARα, β, and γ) and the retinoid X receptors (RXRα, β, and γ). Like VDR, these belong to the steroid/thyroid hormone nuclear receptor superfamily.[75,77] Regulation of gene expression is complex since the RAR and RXR receptors have very different ligand binding specificities. *all-trans*-RA binds exclusively to the RARs, while 9-*cis*-RA binds both receptor types. Induction of gene expression by RA requires an RAR−RXR heterodimer[78] in which the RXR partner is generally silent.[79,80] The retinoic acid response elements (RAREs) of RA-responsive genes consist of a direct repeat of the hexamer sequence 5′-AGG/TTCA-3′ separated by two (DR2) or five (DR5) base pairs or slight variants of this arrangement.[77] The RXRs appear to function largely as required heterodimer partners for other nuclear receptors.

Initial studies in psoriasis using either topical or oral *all-trans*-RA were not very promising because of unacceptable skin irritation and lack of efficacy (see ref 11). The second generation of aromatic retinoids, etretinate (tigason) and acitretin, were effective systemic therapies but ineffective topically for the treatment of psoriasis. These drugs are contraindicated in women of childbearing age because of teratogenicity.[81] Widespread and reversible side effects associated with their use include mucocutaneous irritation, such as dryness and flaking of the skin, as well as elevation of serum triglycerides, which can generally be controlled with lipid-lowering drugs.[82] Systemic retinoids also cause longer-term effects (>1 year) such as osteoporosis or hyperostosis that appear to be irreversible,[81,83] and consequently they are used only for severe forms of psoriasis.

Tazarotene (AGN 190168), a synthetic retinoid of the diarylacetylene class, is a relatively selective ligand (5−10-fold) for the RAR receptors β and γ as compared to RARα. Tazarotene (Tazorac in the United States and Zorac in other countries) has been approved as a topical therapy for the treatment of mild to moderate plaque psoriasis, which constitutes the majority of psoriasis cases.[11] In a double-blinded, placebo-controlled phase III study (n = 324), once daily tazarotene (0.1% or 0.05%) gel or vehicle gel was administered to patients with mild-to-moderate plaque psoriasis for 12 weeks. These patients were monitored for another 12 weeks posttreatment. Treatment success rates ranged from 60−70% in the target lesions of tazarotene-treated patients. Interestingly, the clinical response continued during the 12-week posttreatment observation period.[84] The side effects of tazarotene were mainly limited to local irritation.



Tazarotene

A. Molecular Mechanisms of Tazarotene Action in Psoriasis. Retinoids inhibit the expression of a number of genes that are associated with abnormal or psoriatic differentiation, cell proliferation, and inflammation. Some of these genes contain AP1 or NF-IL6 as their major enhancer factor, and retinoids inhibit their expression by antagonizing the enhancer action of these transcription factors.[85−88] The retinoid-suppressible genes include metalloproteases (stromelysin-1, collagenase, and gelatinase), protooncogenes and transcription factors (c-fos, c-myc, and oct-3/4), growth factors, cytokines and their receptors (TGF-β1, EGF-R, IL-6, and IL-6 receptor), markers of epidermal keratinocyte differentiation (keratins K5, K6, K14, and K16, TGase I, loricrin, MRP-8, and SKALP), and proinflammatory proteins (JE/MCP-1, iNOS, TNF-α, IL-2, IL-6, IL-8, and MRP-8).[89] Tazarotene inhibits AP1- and NF-IL6-dependent gene expression through all three RAR isoforms.[86,88]

Tazarotene appears to have beneficial effects on all three major manifestations of psoriasis, namely keratinocyte proliferation, abnormal keratinocyte differentiation, and immunocyte infiltration in epidermis and dermis. Psoriatic lesions express several genes that are either absent or expressed at very low levels in normal or nonlesional psoriatic skin. Tazarotene inhibits the expression of a number of these genes, including ODC, TGase I, MRP-8, SKALP, K6, K16, HLA-DR, and ICAM-1 in psoriasis and various skin-based systems.[11,90] Further, the level and pattern of expression of filaggrin in psoriatic lesions was normalized by topical tazarotene.[11] Tazarotene also inhibits the IFN-γ-induced expression of MRP-8 and TGase I in keratinocytes.[91] Tazarotene induces the expression of three novel genes, tazarotene-induced gene 1 (TIG1), TIG2, and TIG3, in various skin systems including psoriatic lesions.[79,92,93] The most interesting of these, TIG3, was found to be a novel class II tumor suppressor or antiproliferative gene.[93]

In addition to treating psoriasis, topical calcipotriol and topical tazarotene are effective in treatment of

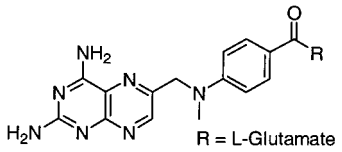
congenital diseases of epidermal keratinization and differentiation, such as certain ichthyoses,[94,95] which are also characterized by epidermal flaking and scaling. Such clinical findings raise the possibility that normalization of epidermal differentiation may be critical to the antipsoriatic activity of both these compound classes. It is interesting, however, that the differences in the effects of retinoids and vitamin D_3 analogues on cultured keratinocyte differentiation far outnumber the similarities: for example, retinoids inhibit the expression of the differentiated phenotype while vitamin D_3 analogues enhance it.[45,91,96] Conceivably, a common gene or set of genes regulated by both classes of ligand may be identified that represents a critical target for reversing abnormal differentiation in the disease.

UV Light

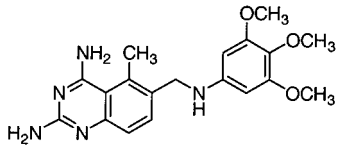
UV light is one of the oldest and most widespread therapies for psoriasis.[25] UVB light (280−320 nm) used by itself or as UVB narrow-band light (at 312 nm) can induce a long-term remission of psoriasis.[9] UVB therapy has the intriguing property that it induces rapid depletion of intraepidermal T-cells from the skin.[23] The reduction in T-cell number appears to precede changes in epidermal differentiation as indicated by the later down regulation of psoriasis markers such as K16. In addition, intraepidermal T-cells undergo rapid apoptosis following narrow-band UVB treatment.[9] Recent studies suggest a likely mechanism. UV irradiation of skin induces epidermal keratinocyte expression of the Fas ligand, CD95L, which in turn can trigger apoptosis in neighboring cells, such as T-cells, that express CD95 or Fas, the receptor for CD95L. For example, UV-treated cultured keratinocytes, which express CD95L, induce apoptosis in T-lymphocytes bearing CD95.[8] A similar series of events is proposed for T-cells in the UV-irradiated psoriatic plaque. It is well-established in mouse that UV irradiation of skin is highly immunosuppressive. UV-mediated immunosuppression does not occur in knockout mice lacking CD95L, indicating that T-cell apoptosis is a central element in the cutaneous actions of UV light.[97]

Methotrexate

Oral methotrexate (MTX) has been widely used for the treatment of psoriasis since the 1960s.[98] One drawback of MTX use is increased risk of cirrhosis of the liver after a cumulative dose greater than 1.5 g, and periodic liver biopsies are recommended after this dose has been reached.[1,98] MTX inhibits the enzyme dihydrofolate reductase (DHFR) and thereby blocks the regeneration of tetrahydrofolate, a critical methyl donor for nucleotide biosynthesis. Because it suppresses DNA synthesis, MTX has been widely used for cancer therapy. It also has antiinflammatory and immunosuppressive effects[7] that may be relevant to its effectiveness in psoriasis as well as rheumatoid arthritis. For example, MTX stimulates the release of adenosine from cells, leading to adenosine-dependent antiinflammatory activity.[99] In addition, MTX induces apoptosis in dividing lymphocytes by a route independent of adenosine and CD95.[7] Apoptosis is not induced in resting T-cells, indicating that MTX may specifically remove T-cell clones recently stimulated by antigen, consistent with its immunosuppressive activity and application to graft rejection.[7] The pharmacodynamics of MTX are complex, and it can inhibit more than one intracellular enzyme: upon diffusion into the cell, MTX is modified by polyglutamylation, trapping MTX within the cell and rendering it a more potent substrate for several enzymes in addition to DHFR, including two enzymes involved in de novo purine synthesis.[99,100] Other inhibitors of DHFR, including trimetrexate, have been used to treat cancer, but their effects in psoriasis have not been reported.[100]



Methotrexate (MTX)



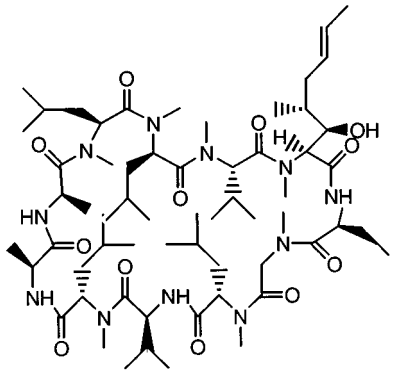
Trimetrexate (TMQ)

Macrocyclic Immunosuppressive Agents

Immunosuppressive agents of the macrocyclic class, first developed for organ transplantation, block IL-2 production and thereby inhibit T-cell growth. These agents bind cytosolic proteins called immunophilins that then become specific inhibitors of the phosphatase calcineurin. Calcineurin is required for the activation and nuclear transport of the transcription factor NF-AT that induces expression of IL-2 mRNA.[101,102] Inhibition of calcineurin blocks the activity of NF-AT, which, in turn, suppresses IL-2 production as well as T-cell activation.[103] Cyclosporine A binds cyclophilin, whereas tacrolimus (FK506) and the ascomycins bind to a different immunophilin called macrophilin 12 or FK-binding protein-12 (FKBP-12).[29]

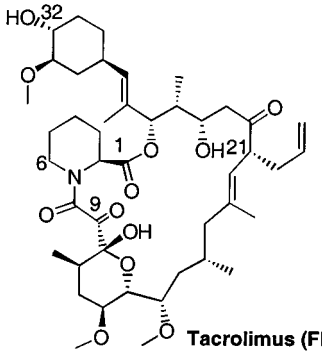
Cyclosporine A. Cyclosporine A (CsA) is a cyclic polypeptide of fungal origin that was described in 1972. The effects of oral cyclosporine in psoriasis were fortuitously discovered in 1979 from an observation that plaque-type psoriasis cleared in all four patients who received the drug as an immunosuppressive agent for psoriatic arthritis.[104] Subsequent clinical trials using a 2.5−5 mg/kg/day oral dosage further confirmed the efficacy of CsA in the treatment of various types of psoriasis, including erythrodermic and plaque-type.[105] Systemic CsA can produce reversible nephrotoxicity and susceptibility to viral infection. Efforts to treat psoriasis with topical cyclosporine preparations have failed, owing probably to its large size (1202 Da) and consequent poor skin penetration.[106] On the other hand, intralesional injections of CsA were found to be clinically effective in a double-blind study involving a total of 31 patients with plaque-type psoriasis.[107] Systemic CsA rapidly suppresses the proinflammatory cytokines IL-8 and Gro-α in the psoriatic plaque (within 1 week) but has no effect on inducible expression of these cytokines in cultured keratinocytes, suggesting that CsA is a fairly specific inhibitor of T-cell cytokine expression and that IL-8 and

Gro-α down-regulation in psoriasis (presumably at the level of the keratinocyte) is an indirect result of CsA on the infiltrating T-cells.[20]



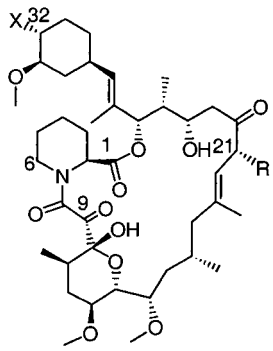
Cyclosporine A

Tacrolimus (FK506, Prograf). Tacrolimus (FK506), a hydrophobic macrolide lactone isolated from *Streptomyces tsukubaensis*, is 10−100 times more potent than CsA in vitro and in vivo.[108] FK506 rapidly clears psoriatic lesions in patients with severe, recalcitrant psoriasis at doses of 0.05−0.15 mg/kg/day.[109] Among the side effects frequently reported with systemic FK506 are diarrhea, paresthesia, and insomnia. In contrast to CsA, topical application of 0.04−0.4% FK506 markedly inhibited the inflammatory hypersensitivity reaction to dinitrofluorobenzene (DNFB) on the skin of domestic pigs.[110] The treatment response was similar to that of 0.13% clobetasole, a potent glucocorticoid. More recently, in a phase II randomized, double-blind study involving 16 patients with chronic plaque-type psoriasis, using a microplaque assay where lesions were descaled with salicylic acid and drug applied under occlusion, 0.3% tacrolimus ointment was found to significantly reduce eythema, infiltration, and superficial blood flow and to decrease epidermal thickness.[111] Significantly, topical macrolide immunosuppressants do not appear to carry the risk of skin atrophy, which is a major drawback associated with corticosteroids.[112]

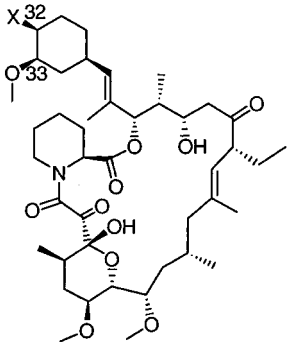


Tacrolimus (FK506)

Ascomycins: Ascomycin, SDZ 281240, SDZ ASM 981, and ABT 281. Other analogues of FK506 were also isolated in fermentations by scientists at Fujisawa.[113] Of these, FR-900520 had been previously isolated by another group and was named ascomycin.[114] As is evident from its structure, ascomycin differs from FK506 only in the side chain at C-21.[115,116] Numerous structure−activity relationship (SAR) studies have been conducted on FK506 and related ascomycin derivatives. In summary, these studies indicate that the "western" side is involved in the interaction with the macrophilin-binding protein FKBP-12, while the "eastern" portion interacts with calcineurin to form a ternary complex.[117] The hydroxy group at C-32, however, does not interact with either of these proteins, permitting structural modifications that alter physicochemical properties but not immunosuppressive activity.



FK 506: X = OH ; R = -$CH_2CH{=}CH_2$
Ascomycin: X = OH ; R = -CH_2CH_3



SDZ ASM 981: X = Cl
ABT 281: X = 1-tetrazolyl

Some of the ascomycin derivatives, SDZ 281240 (structure not disclosed) and SDZ ASM 981, have similar activity to FK506 and have also been tested as topical immunosuppressives. In a randomized, double-blind, placebo-controlled clinical study, 15 patients with severe recalcitrant psoriasis achieved a clearing of psoriasis at concentrations of 0.1% and 1% topical SDZ 281240 after 10 days of treatment.[118] The therapeutic effect of 1% SDZ ASM 981 when applied under occlusion was comparable to that of 0.05% clobetasol-17-propionate, a potent topical corticosteroid.[119] SDZ ASM 981 is also effective in the topical treatment of allergic contact dermatitis (ACD).[120]

Psoriatic skin is characterized by erythema (hyperemia) and increased blood flow.[121] Therefore the risk of side effects induced by systemic absorption of topical agents such as the immunosuppressives is increased. Synthetic ascomycin derivatives have been made with the specific aim of rapid clearance in systemic circulation while retaining local immunosuppressive activity. Of these analogues, ABT-281 is an immunosuppressant equivalent to FK506 in potency when applied topically but with dramatically lower (about 10−100-fold) immunosuppressive potency when given systemically.[117] ABT-281 thus has a promising profile as a topical agent for the treatment of psoriasis and related diseases such as atopic dermatitis.

As noted above, several topical macrolide immunosuppressants are therapeutically effective in psoriasis when applied under occlusion after the psoriatic scale is stripped off the plaque. Because these procedures are not compatible with widespread topical application, the design of formulations that provide good topical penetration without occlusion is probably critical for the clinical success of this class of agents.

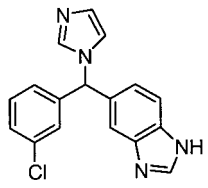
Fumaric Acid Esters

A mixture of methyl and ethyl esters of fumaric acid has been used for many years in Germany as an oral therapy for psoriasis, and a specific mixture of fumaric acid esters (fumaderm) is available commercially. A

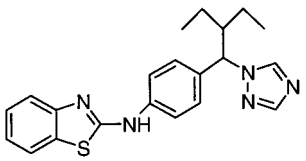
clinical response requires several months of treatment.[37] The dosage employed (1.2 g/person/day) suggests either that the fumaric acid esters have a low-affinity therapeutic target or that a specific metabolite is the ultimate active form. B- and T-lymphocyte levels are slightly reduced during the course of therapy, but the significance of this is not known.[37]

Experimental Therapeutic Classes

Cytochrome P-450 Inhibitors: Liarozole. Since retinoids are well-established therapies for the systemic treatment of psoriasis, inhibition of the breakdown of endogenous RA has been explored as an alternative therapeutic approach. RA is metabolized by a cytochrome P450-dependent pathway to 4-hydroxy- and 4-oxoretinoic acid, and these metabolites are further modified by glucuronidation and are rapidly eliminated. The recently described cytochrome P450 CYP26 or P450RAI (P450 RA-inducible) is a highly specific RA metabolizing monooxygenase[122] whose expression is dramatically induced by retinoids. Liarozole (R 75251), an imidazole derivative, inhibits CYP26 and several other cytochrome P450s and is also able to mimic the antikeratinizing effects of RA in vivo.[123] A double-blind, randomized clinical study involving 20 patients with severe plaque-type psoriasis was conducted where half of the patients were treated with oral liarozole (75 mg, twice daily) and the other half were treated with oral acitretin (25 mg/day).[124] After 12 weeks of treatment, both groups responded with a similar decrease in the PASI score from ~20 to ~10. Liarozole was found to modulate cell biological parameters in lesional and uninvolved skin in a manner that was similar to acitretin. For example, there is a decrease in the CD 11b-positive cell population (neutrophils, monocytes, and macrophages) and a decrease in epidermal ICAM-1 expression.[125] The side effects of liarozole include those of hypervitaminosis A.[125] Recently, more potent inhibitors in this class have been identified, such as R 115866.[126] It remains to be determined whether a topical CYP26 inhibitor will be effective in the treatment of psoriasis.



Liarozole



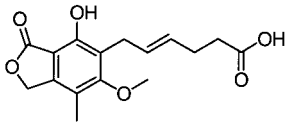
R 115866

Immunosuppressive Biological Macromolecules. T-cell activation is exquisitely regulated by both cell−cell interactions and specific growth factors, and T-cell function is central to regulation of a system designed to suppress recognition of autoantigens and induce a vigorous response to foreign macromolecules. Several therapeutic macromolecules that can block or subvert critical receptors required for T-cell activation have been tested for the treatment of psoriasis in recent years. A fusion protein of IL-2 and diphtheria toxin, DAB_{389}IL-2, containing the enzymatically active region of diphtheria toxin, clearly has a beneficial effect in cases of psoriasis that are recalcitrant to other therapies[30] but has suffered from unpleasant side effects at higher doses (fever, chills, nausea) and less than remarkable efficacy.[127] This innovative therapy appears to act analogously to the macrocyclic immunosuppressants such as CsA and FK506 by inhibiting the growth of T-cells most dependent on IL-2. Another approach has been to block a costimulatory signal required for T-cell clonal expansion in response to presentation of specific antigens by antigen-presenting cells (APCs). The T-cell surface protein CTLA-4 (cytotoxic T-lymphocyte-associated antigen-4) is a required accessory molecule for T-cell receptor activation. CTLA-4 binds to the B7 family of molecules (CD80 and CD86) on the APC surface, ensuring that the T-cell responds in the proper context. APCs that may be involved in psoriasis pathogenesis include infiltrating macrophages and epidermal Langerhans cells which, when activated, express markers of the B7 class.[36] CTLA-4 consists of two proteins, CD28 and CD152. A fusion protein, CTLA4Ig (BMS 188667), which consists of the constant region of a human immunoglobulin with a portion of CD152, has recently been successfully tested as a systemic therapy for psoriasis[3]. CTLA4Ig blocks the interaction of APCs and T-cells and is immunosuppressive in animal models. Doses of CTLA4Ig in the range of 5−40 mg/kg reduced both epidermal hyperplasia and T-cell infiltration into the epidermis.[3] These findings on the role of T-cell activation are consistent with earlier trials of antibodies to CD4, a T-cell surface marker, which are also effective in treatment of psoriasis. OKT(R)cdr4a, for example, a humanized form of a mouse monoclonal antibody to CD4, was therapeutically effective in a group of 6 patients given a single course of treatment at 1 mg/kg/day for 6 days.[128] Although these studies provide strong evidence for a central role for T-cells in the pathogenesis of psoriasis, it remains to be seen whether the particular therapies will be readily applicable in routine clinical practice. More recently, subcutaneous injection of IL-10 in relatively small amounts, about 20 μg/kg as opposed to the larger doses of CTLA4Ig and CD4 antibody, caused significant improvement of psoriasis in open label studies.[4,27] The clinical pharmacology of IL-10 action supports the conclusion that type 2-cytokine expression is induced, consistent with suppression of cell-mediated immunity. IL-11, which suppresses type 1 cytokine expression, has also been suggested to be therapeutically effective.[28] Finally, a humanized antibody to CD11a, a subunit of the leukocyte function-associated antigen (LFA-1), blocks migration of T-cells to the psoriatic plaque and also has a significant therapeutic effect when given intravenously.[129]

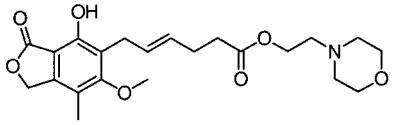
IMPDH Inhibitors: CellCept and VX-497. Inositol-5-monophosphate dehydrogenase (IMPDH) is essential for the de novo biosynthesis of guanosine nucleotides in T- and B-cells. It catalyzes the nicotinamide adenine dinucleotide (NAD)-dependent oxidation of inosine-5′-monophosphate (IMP) to xanthosine-5′-monophosphate (XMP). Unlike other cells that have alternate enzyme pathways available, the proliferation of these lymphocytes totally depends on the IMPDH pathway. Therefore T- and B-cells are very sensitive to IMPDH inhibition, and this enzyme appears to be a good target for immunosuppressive drugs. Increased IMPDH activity has been demonstrated in leukemic cell lines and

tumor tissues. Since psoriasis involves proliferation of T-lymphocytes in the epidermis and dermis, this disease has been identified as a potential therapeutic target of an IMPDH inhibitor.[130]

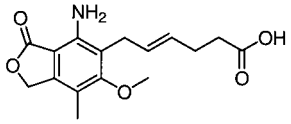
Mycophenolic acid (MPA), an antibiotic isolated from the fermentation broth of several *Penicillium* species, was found to exhibit antiproliferative activity on a variety of tumors in mice and rats by functioning as a potent, uncompetitive, reversible IMPDH inhibitor. MPA was also used as an oral agent in the treatment of psoriasis with good to excellent responses but with some serious side effects.[131] Mycophenolate mofetil (MMF, CellCept), the morpholinoethyl ester prodrug of MPA, was approved recently for use as an immunosuppressive agent for acute kidney transplant rejection. A recent report suggests that MMF is most effective in psoriatic arthritis among patients tested.[132]



MPA



MMF



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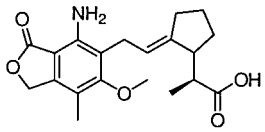

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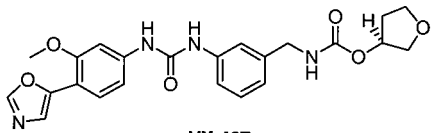
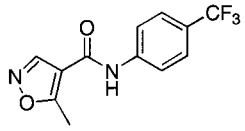


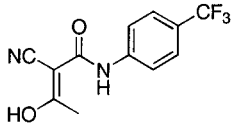
VX-497

The solution of the X-ray crystal structure of MPA-bound IMPDH[130] led to the discovery of the VX-497 class of second-generation IMPDH inhibitors.[133] VX-497 is currently under development as an immunosuppressant for psoriasis and other diseases. MPA inhibits the enzyme by simultaneously mimicking the nicotinamide portion of the NAD co-factor and a catalytic water molecule. The lipophilic portion of MPA (including the hexenoic acid tail, the methoxy group, and the methyl group) makes van der Waals contacts with the side chains of amino acid residues in the active site, while the phenolic hydroxyl group forms hydrogen bonds with Thr-333 and Gln-441 residues. These hydrogen bonds are very important for inducing a tight fit within the MPA−IMPDH complex. MPA is quite potent (IC_{50} = 0.02 μM) in an enzymatic assay for IMPDH inhibition. Dehydroxy-MPA, which cannot form the hydrogen bonds with Thr-333 and Gln-441, is a 1000-fold less potent than MPA. Furthermore, the amino-substituted analogue, **1**, is about 8-fold less potent (IC_{50} = 0.154 μM) due to disruption of one of these hydrogen bonds. The hydrogen bond between the carboxylic acid of MPA and Ser-276 of IMPDH also contributes significantly to the binding since the corresponding ester and alcohol are 50-fold less potent in IMPDH inhibition. The cyclopentane ring-locked analogue, **2**, and VX-497 are two of the most potent IMPDH inhibitors (IC_{50} = 7−8 nM) described in the literature.

Leflunomide, a Immunosuppressant and Nucleotide Synthesis Inhibitor. Leflunomide (brequinar sodium) inhibits pyrimidine synthesis in lymphocytes and has an immunosuppressive effect in animal models of graft rejection. Recently, leflunomide (20 mg/day) was shown to be as efficacious as MTX (7.5−15 mg/week) in treatment of rheumatoid arthritis in a large clinical trial.[134] The possibility that leflunomide is also active in psoriasis must be seriously considered.[135] A metabolite of leflunomide, A77 1726 or RS-61980, blocks growth of a T-cell lymphoblastoma in the concentration range of 1−10 μM, and this inhibition is relieved by addition of exogenous uridine. Consistent with its inhibition of de novo pyrimidine biosynthesis, A771726 blocks dihydroorotate dehydrogenase with a K_i = 2.7 μM.[136] Its potency in cell-based assays of growth inhibition appears to be approximately one-tenth that of MTX.[7,136]



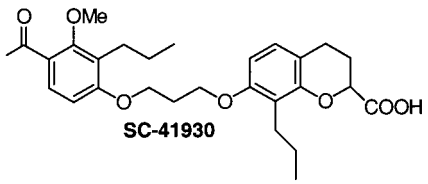
Leflunomide



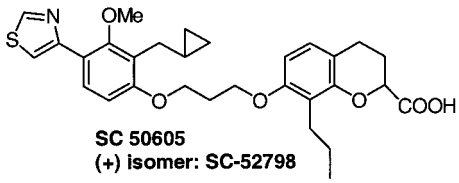
A77 1726 (RS-61980)

Leukotriene B_4 Antagonists: SC-52798, VML295 (LY293111), and ZK 158252. Psoriatic lesions are characterized by the accumulation of polymorphonuclear leukocytes (PMNs). Leukotriene B_4 (LTB_4), a 5-lipoxygenase-derived metabolite of arachidonic acid, and other metabolites of arachidonic acid are found in elevated concentrations in psoriatic skin.[137] LTB_4 is involved in the migration, chemotaxis, and degranulation of PMNs.[138] Considerable efforts have been directed toward LTB_4 receptor antagonists in the search for novel therapeutic agents for inflammatory diseases. SC-52798, the optically pure dextra rotatory isomer of SC-50605, is a second-generation LTB_4 antagonist based on an earlier compound, SC-41930. SAR studies showed that the introduction of the thiazole group in SC-52798 enhanced the in vitro potency of inhibition of LTB_4-induced PMN chemotaxis and degranulation ~20−25-fold in comparison with the methyl ketone analogue, SC-41930.

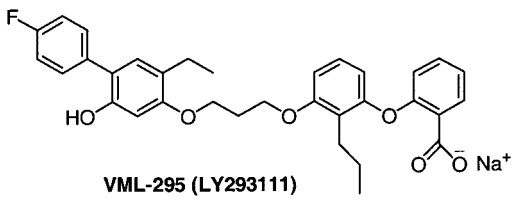
VML-495 (LY293111) is closely related to SC-52798 in structure. In normal males, oral VML-495 twice daily (200 mg/dose) blocked epidermal hyperplasia and PMN infiltration induced by topically applied LTB_4.[139] A placebo-controlled clinical study was conducted on patients with stable plaque-type psoriasis using the same dosing regimen over a 4-week period.[138] The treatment was well-tolerated, and the expression of the LTB_4-inducible PMN marker CD11b on peripheral blood PMNs from psoriatic patients was almost completely blocked in the VML-495-treated group. However, there was no significant reduction in the severity of psoriasis, nor was there any effect on histological parameters of inflammation and epidermal proliferation.[138]



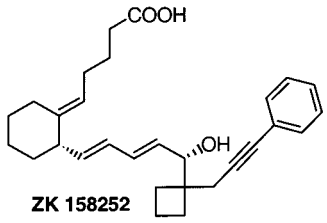
SC-41930



SC 50605
(+) isomer: SC-52798



VML-295 (LY293111)

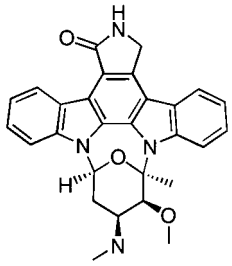


ZK 158252

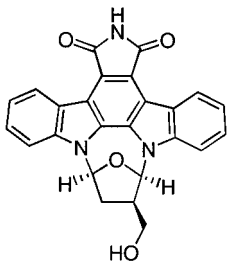
ZK-158252[140] is another LTB_4 antagonist that inhibits LTB_4-induced chemotaxis of human neutrophils, and it inhibited edema and neutrophil infiltration induced by topical LTB_4 in a guinea pig model.[141] Despite its efficacy in animal models, in a randomized, double-blind study on 69 patients with psoriasis, ZK 158252 as a 3% alcoholic topical solution used once daily for 10 days had no clinically relevant effects on the expansion, erythema, thickening, and scaling of psoriatic lesions.[142] These results raise serious doubts about the role of LTB_4 in the pathogenesis of psoriasis.

Antisense Oligonucleotides. A major T-cell adhesion molecule, ICAM-1, is upregulated in psoriatic epidermis, and its expression can be strongly induced in human keratinocytes by treatment with IFN-γ.[21] A direct approach to down-regulation of this molecule involves destabilization of its mRNA by formation of a mixed hybrid with an antisense oligonucleotide that is stable and permeable to the cells. Because the RNA−DNA hybrid is very sensitive to intracellular nucleases, ICAM-1 mRNA can be degraded before it is translated into protein. One such ICAM-1-inhibitory antisense oligonucleotide, ISIS 2302, has a modified backbone that enhances its half-life in vivo. Initial clinical data have suggested that ISIS 2302 is effective as an intravenous formulation for an inflammatory disease of the bowel mucosa, Crohn's disease,[143] but these findings have not been reproducible and clinical trials are continuing. Notwithstanding these data, antisense oligonucleotides such as ISIS 2302 may have potential as a treatments for psoriasis because of their potential for targeted repression of gene expression.

Protein Kinase Inhibitors. Protein kinase C (PKC) regulates normal keratinocyte growth and differentiation, and its dysregulation is suspected to be involved in the inflammation and abnormal differentiation observed in psoriasis.[144,145] Staurosporine, a naturally occurring indolocarbazole, is one of the most potent PKC inhibitors yet reported, but it appears to be relatively nonselective[146] since it also inhibits cAMP-dependent kinases and protein tyrosine kinases (PTKs). A variety of synthetic staurosporine derivatives have been designed to improve the selectivity of PKC inhibition.[147,148] SCH 47112 is a novel synthetic indolocarbazole that inhibits PKC in vitro at nanomolar concentrations (IC_{50} = 1.7 nM) compared with IC_{50} = 7 nM for staurosporine.[149] Replacement of the imide nitrogen in SCH 47112 to form a ring-expanded hydrazide or N-methylation leads to complete loss of inhibitory activity while changes in the "southern" region are well-tolerated. However, in general, these analogues are specific neither for individual PKC isozymes nor for PKC versus other serine/threonine protein kinases. SCH 47112 potently inhibits inflammation and hyperplasia in hairless mouse skin induced by the natural PKC agonist 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as well as TPA-induced differentiation in cultured human keratinocytes.[150] However, the importance of PKC activation in psoriasis has not been tested through appropriate studies with these inhibitors.



Staurosporine

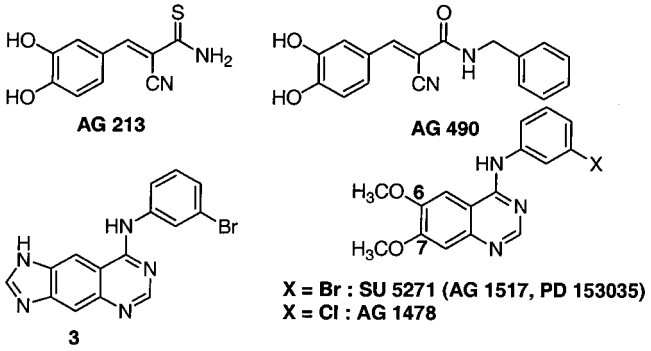


SCH47112

PTK Inhibitors: AG 213, AG 555, and SU 5271. Elevated activation or overexpression of ligand-activated PTKs is implicated in cancers and also in several nonmalignant diseases such as psoriasis.[16,17,151] Many PTKs are growth factor receptors, such as the EFGR, or are closely associated with growth factor signaling. Others are involved in the regulation of angiogenesis and the immune response.[152] Ligands for PTKs abundantly overexpressed in psoriasis include TGF-α, amphiregulin, and heparin-binding EGF, all ligands for EGFR,[16,17,151] and cytokines such as GM-CSF.[22] The possibility that hyperactivation of EGFR is the cause of epidermal hyperproliferation in psoriasis is at least part of the reason why agents that block EGFR have been extensively studied.[153]

In general, PTK inhibitors can be pure competitors with either ATP or the protein substrate, or bisubstrate inhibitors. Achieving selectivity of the PTK inhibitors for EGFR versus other PTKs such as IGFR (insulin-like growth factor receptor) and PDGFR (the platelet-derived growth factor receptor) is important. The core structures of the first generation, small-molecule tyrphostins (tyrosine phosphorylation inhibitors), were initially based on the structure of tyrosine itself, using 3,4-dihydroxy-*cis*-cinnamonitrile as the basic pharmacophore. These compounds were not very specific for EGFR because they also inhibited either PDGFR or the HER2-Neu protooncogene that is often overexpressed

in breast cancer. The tyrphostins AG213 and AG490 inhibit ($IC_{50} = 7-15\ \mu M$) the EGF-dependent proliferation of human and guinea pig keratinocytes and also reversibly induce their growth arrest,[154,155] substantiating the growth inhibitory potential of this class of compounds.



Small-molecule inhibitors belonging to structural classes such as the anilinoquinazolines, pyridopyrimidines, pyrrolopyrimidines, and pyrazolopyrimidines can have much higher potency and specificity toward EGFR.[156] The synthetic molecule SU 5271 (AG 1517, PD153035) ($IC_{50} = 0.025$ nM) belongs to the quinazoline class of PTKs that are competitive inhibitors of ATP and are highly selective for EGFR. The chloroaniline-condensed derivative AG 1478 is >1000−10000-fold selective for the EGFR versus HER2-Neu and PDGFR.[154] The 6,7-unsubstituted linear imidazoquinazoline **3** was shown to be $>10^6$ fold selective for the EGFR versus PDGFR, fibroblast growth factor receptor, and insulin receptor.[157] Replacement of the 6,7-dimethoxy groups in AG1517 with 6,7-diethoxy groups increases the potency 4-fold.[158] Most other changes have proven detrimental to the potency of inhibition. SU5271 has been in phase I clinical trials since early 1997 for the topical treatment of plaque-type psoriasis.[159] The insoluble nature of anilinoquinazolines such as SU-5271[160] may seriously compromise their ability to penetrate into the epidermis, however, reducing the chances of observing topical efficacy in psoriasis.

Novel Pharmacological Approaches to Psoriasis Therapy

Nuclear Receptor Ligands. Rapid advances in the field of nuclear hormone receptors in the last 15 years have led to the identification of biochemical targets for three major psoriasis therapies: glucocorticoids, analogues of vitamin D_3, and retinoids. It is too soon for new drugs to have emerged based on an improved understanding of the biology of their target receptors (GR, RAR, and VDR), yet recent findings in the nuclear receptor field suggest that new classes of ligands may be identified which are "functionally selective" or capable of modulating some receptor functions but not others. For example, antagonists of two nuclear receptor classes, the RARs and the estrogen receptors (ERα and ERβ), induce agonist-like effects in a restricted range of tissues and cell types. The ER antagonist raloxifene is used in the treatment of osteoporosis, as estrogen is, but has no trophic effects on mammary and uterine tissue.[161,162] Raloxifene and other ER antagonists are also used clinically to prevent the growth of estrogen-dependent breast cancers. These novel estrogen ligands are referred to as SERMs, or selective estrogen receptor modulators.[161,163]

We have recently described a class of highly potent RAR antagonists, including AGN 193109,[164] which suppress basal transcriptional activity at the RARs and which are therefore referred to as retinoid inverse agonists.[165] Surprisingly, two gene products suppressed by retinoid agonists in cultured human epidermal keratinocytes, MRP-8 and stromelysin-1, are also inhibited by AGN 193109. Another class of retinoid antagonists, referred to as neutral antagonists, has no effect on MRP-8 regulation and does not inhibit basal transcriptional activity from the RARs as inverse agonists do.[165,166] Competition studies demonstrate that agonists and inverse agonists suppress MRP-8 by different mechanisms through the same receptor, RARγ. Since MRP-8 and stromelysin-1 may be involved in inflammation, these findings open up the possibility that this family of retinoid inverse agonists[167] will have novel and useful properties in the treatment of inflammatory diseases such as psoriasis.

Nuclear receptors act in conjunction with a wide range of co-regulatory molecules, known as co-activators and co-repressors, whose binding to receptor is regulated by agonist or antagonist, respectively.[168] The tissue selectivity of estrogen antagonist activity may depend on cell-restricted expression of the different nuclear receptor co-activators and co-repressors.[163] In the case of the RARs, inverse agonists can be distinguished from neutral antagonists by increased recruitment of the negative co-regulator or co-repressor, N-CoR.[169] The differential recruitment of N-CoR by inverse agonist requires RAR heterodimerization with RXR on an appropriate DNA response element, indicating that multisubunit protein allosteric interactions may be essential for distinguishing the two types of ligands. Understanding of subtle ligand-dependent changes in the co-activator and co-repressor binding sites on receptors may facilitate identification of new function-selective ligands. For example, peptide analogues of co-activators have been designed which can distinguish between the co-activator binding surface of ER when bound to one or the other of two closely related SERMs, tamoxifen and raloxifene.[170]

Selective modulators of GR and VDR have been identified but not extensively characterized. In ideal form, such modulators for GR or VDR, also referred to as "dissociated ligands", will inhibit gene expression by interfering with enhancers such as AP-1, NF-IL6, and NF-κB that are linked to hyperproliferation and inflammation but will not induce expression of genes thought to be linked to common side effects. Candidate dissociated glucocorticoids have been recently described which behave as antagonists for GR-mediated induction of gene expression in some but not all cell types tested and retain the ability to antagonize AP-1 activity and inhibit NF-κB signaling.[171,172] These compounds also have antiinflammatory activity in vivo, but it is not known whether GR-mediated side effects such as skin atrophy have been eliminated. The VDR antagonism of certain recently described vitamin D_3 analogues are also cell type-specific, but the in vivo properties of these have not yet been explored.[173] Although these data demon-

strate that modulators of VDR and GR can be synthesized which are cell-type-selective in their agonist or antagonist activity, it is so far unclear whether they have desirable or unique properties in vivo consistent with an improved therapeutic index.

Other approaches to increasing therapeutic selectivity and safety for nuclear receptor ligands include the design of features that enhance metabolism or elimination, as has been done successfully for vitamin D_3 analogues, or the design of receptor subtype-selective ligands, which is applicable to the RARs. Current therapeutic retinoids for psoriasis bind all three RARs, but newer receptor-selective retinoids for RARγ and RARα have been described.[174−176] RARγ is the major subtype in skin, and it is also clearly the critical receptor for topical irritation in rodents.[174,177] It remains to be determined whether true RARγ-specific retinoids will exhibit an improved therapeutic index in the treatment of psoriasis. RARα-specific retinoids neither cause topical irritation nor regulate differentiation in human keratinocyte cultures, so their potential cutaneous function is unclear.[166,177] Because RARα is prominent in hematopoeitic differentiation,[178] however, it is possible that the recently characterized RARα-specific retinoids (agonists and antagonists[179]) will have therapeutic benefits in psoriasis.

GR, VDR, and RAR are in fact members of a much larger family of nuclear receptors,[180] of which additional members have the potential to be therapeutically important in psoriasis. Preliminary data suggests that PPARγ agonists of the thiazolidinedione or TZD class may be effective. These compounds are not only widely used as a novel therapy for adult onset (type 2) diabetes, but they can also reduce disease severity in a mouse model of inflammatory bowel disease[181] and inhibit proinflammatory enzymes such as inducible nitric oxide synthase.[182] Two recent open-label studies, in which a total of 8 patients were examined, demonstrated significant therapeutic improvement during therapy with the TZD troglitazone.[183,184] There was also a substantial inhibition of epidermal keratinocyte growth in cell culture, raising the possibility that members of this class could be active topically.[184] Among the remaining nuclear receptors, many are so-called orphans which do not have identified high-affinity ligands.[180] Some of these, such as RORγ, regulate T-cell development and thus have potential as targets which may regulate T-cell participation in autoimmunity.[185]

Inhibitors of Cytokine and Growth Factor Action. There currently are no psoriasis therapies that explicitly target epidermal hyperplasia, and thus there are no growth factor or cytokine receptors, other than those involved in T-cell activation, which can legitimately be considered therapeutic targets. This is true even in the case of EGFR, which is the target of several growth factors highly elevated in the psoriatic plaque:[16,17] agents which block EGFR tyrosine kinase activity are clinically unproven so far. A large number of additional cytokines and growth factors cause some degree of psoriasis-like epidermal hyperplasia and inflammation when overexpressed in transgenic mice, but these disease models are of uncertain validity.[32,186] The best approach to modeling psoriasis is a cumbersome one: grafting of uninvolved patient skin to the severe combined immunodeficient (scid) mouse.[187] Exogenously activated T-cells are injected to induce a realistic plaque, which is responsive to some but not all of the immunosuppressive therapies used successfully to treat psoriasis.[32]

Because the evidence that T-cells have a central role in psoriasis pathogenesis is so strong, it is likely that all novel immunosuppressive agents will be tested in the disease. The side effect of global immunosuppression has so far limited application to the most severely afflicted, however. An alternative is to design agents which inhibit specific steps of T-cell activation taking place primarily in skin. The approach is appealing since the epidermis and associated microorganisms may be the source of antigens stimulating T-cell autoreactivity.[188] In addition, such therapeutics could be applied topically and would be considerably safer, just as the topical macrocyclic immunosuppressives appear to be. For example, clinical evidence suggests that Langerhans cells, the specialized dendritic APCs residing in the epidermis, are closely involved in T-cell activation in psoriasis.[36] These cells process antigen for presentation to the T-cells and express CD80 and CD86, the costimulatory molecules of the B7 family required for T-cell activation. Although CTLA4Ig, which binds to CD80 and CD86 (B7-1 and B7-2) and causes some improvement in psoriasis with systemic treatment,[3] is thought to have some critical systemic effects, the authors of this study also suggest that more localized therapy could be effective. Ligand binding to CD95 or Fas appears to be well-validated as a mechanism of inducing epidermal T-cell apoptosis based on studies of the action of UV light.[8,97] The holy grail of combinatorial chemistry, the design of small molecules which can mimic receptor recognition by large polypeptides,[189] may provide the critical avenue for developing novel, topically available therapies to modulate these cell−cell interaction pathways. The application of tyrosine kinase inhibitors may also be expanded: for example, the tyrosine kinase JAK3 is associated with IL-2 receptor signaling, and the fact that human mutations in this gene cause serious immunodeficiency suggests that it also may be an excellent target for psoriasis therapy.[190] Finally, promising therapies such as PTK inhibitors and macrolide immunosuppressives are limited by the ability of molecules to penetrate the epidermis. This in itself is a major challenge to the medicinal chemist.

Conclusions

Because of the complexity of its pathogenesis, the primary if not exclusive source of new paradigms in psoriasis therapy to date has been clinical observation and clinical research rather than basic research. Nonetheless, now that pathways related to nuclear receptor or T-cell activation have been clearly defined, additional targets may be selected based on these models. In addition, it can be assumed that successful drugs to these targets may have applications in other hyperproliferative and inflammatory diseases, including arthritis, inflammatory bowel disease, and cancer.

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