

# Immunomodulatory Effects of Vitamin D Analogues in Psoriatic Skin

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Psoriasis is an inflammatory skin disease characterized by hyperproliferation and incomplete differentiation of keratinocytes. Mounting scientific evidence suggests that this epidermal alteration occurs as a response to an immunologic injury, giving rise to the concept that psoriasis is a skin-specific autoimmune disease. Indeed, many effective therapeutic agents for psoriasis are immunosuppressive in nature, lending further support to this view. The well known ability of calcipotriene and 1,25(OH)<sub>2</sub>D<sub>3</sub> to inhibit keratinocyte proliferation and to induce its differentiation is certainly compatible with their antipsoriatic actions. In addition, topical calcipotriene has been shown to correct, at least in part, the local cytokine imbalance observed in psoriatic lesions. Interleukin (IL)-8 is a proinflammatory cytokine that is chemotactic for polymorphonuclear cells and T lymphocytes. It also promotes proliferation of keratinocytes and endothelial cells. In lesional psoriatic skin, IL-8 and its receptor levels are markedly elevated. IL-10 is an immunosuppressive cytokine, which as a type2 (T2) cytokine antagonizes cell-mediated immunity. Indeed, IL-10 administration has been shown to improve psoriasis. Topical calcipotriene markedly reduces elevated levels of IL-8 while simultaneously increasing IL-10 levels in lesional skin of psoriasis. These changes occur very early, within the first three days of therapy, prior to significant clinical improvement of psoriasis, indicating that the cytokine alterations are not simply secondary to resolution of psoriatic plaques. Therefore, elaboration of the immunosuppressive cytokine IL-10 and a concomitant reduction in the proinflammatory cytokine IL-8 may mediate the immunopharmacological improvement in psoriasis by calcipotriene. (*Ann Dermatol* 13(4) 201~204, 2001).

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*Key Words* : Psoriasis, Vitamin D<sub>3</sub>, Immunosuppression, Cytokines

Marked epidermal hyperplasia with parakeratotic stratum corneum clearly displays at histologic level, aberrant features of keratinocytes in psoriasis. Cell kinetic studies have revealed that in the hyperplastic epidermis, basal keratinocytes undergo more frequent cell divisions, and transit through the epi-

dermal layer much more rapidly than they do in normal skin<sup>1</sup>. The hyperkinetic epidermis most likely contributes to the formation of parakeratosis, which indicates incomplete differentiation of keratinocytes in the stratum corneum. Given the striking structural and functional alterations in psoriatic epidermis, it follows that drugs that can reduce proliferation and promote differentiation of keratinocytes should improve psoriasis. Vitamin D and its analogues, collectively referred to as deltanoids, represent one such class of drugs. The effectiveness of deltanoids in psoriasis is well established. Calcipotriene (a.k.a. calcipotriol and MC-903) is the most widely available and used member of this group. Both 1,25-dihydroxyvitamin D<sub>3</sub>[1,25

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Received September, 2001.

Accepted for publication September, 2001.

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(OH)<sub>2</sub>D<sub>3</sub>] and calcipotriene have been shown to inhibit proliferation and to induce differentiation of keratinocytes, which are compatible with their antipsoriatic effects<sup>2,4</sup>.

Although psoriasis is a disease characterized by keratinocyte hyperproliferation and altered differentiation, mounting scientific evidence indicates that they are the result of an activated immune response. This view of psoriasis as a disease of epidermal response to an immunologic injury emerged from a serendipitous observation that oral administration of cyclosporin A (CSA) improves psoriasis<sup>5</sup>. Subsequent confirmation of this finding in a double-blind, placebo-controlled trial of CSA in psoriasis<sup>6</sup>, and rapidly accumulating evidence that the primary action of CSA is to inhibit lymphokine release and proliferation of T cells, supported the major paradigm shift. Compatible with this view is the fact that, almost all drugs that have successfully been used or are being experimentally used to treat psoriasis interfere, at some step, in T cell activation. Indeed, the fact that effective pharmacological agents share a common mechanism of inducing immunosuppression has strongly supported the concept that psoriasis is an immune system-mediated disease. Therefore, it is reasonable to speculate that, in addition to its effect on keratinocyte proliferation and differentiation, a mechanism involved in calcipotriene-mediated improvement of psoriasis may also include immuno-modulation. In support of this speculation, 1,25(OH)<sub>2</sub>D<sub>3</sub> and its analogues have been shown to be immunosuppressive in animal models of autoimmune disease, preventing the onset, or retarding the progression, of such diseases<sup>7-9</sup>.

A thoughtfully designed clinical study has been conducted to examine the immunomodulatory effect of calcipotriene in psoriasis. It revealed a temporal relationship between clinical improvement in psoriasis and two sets of relevant laboratory parameters (cytokines and the presence of inflammatory cells) in the lesional skin. Since many abnormal parameters measurable in lesional skin of psoriasis normalize as the lesion improves clinically, knowing the time course is necessary for understanding any causal associations between measured parameters of interest and improvement in psoriasis. In this double-blind, placebo-controlled study, thirty subjects were randomized to receive either calcipotriene (0.005%) or its vehicle ointment twice

daily for 6 weeks<sup>10</sup>. Complete clinical evaluations were made at baseline and days 3, 7, and at weeks 2, 4, and 6. At each visit, three clinical parameters (scale, erythema and thickness) of psoriasis were graded on a scale of 0 to 8, where 0 is none and 8 is the most severe. Consistent with previous studies, after 6 weeks of therapy, the improvement in scale, erythema and thickness was significantly ( $p < 0.05$ ) greater in the calcipotriene group as compared with statistically significant improvement was observed with calcipotriene was at day 7 for scale and erythema, and at day 14 for thickness. At the earlier visit on day 3, no significant improvement in any of the three parameters was observed.

Two skin biopsies were performed at each of the first three visits (baseline, days 3 and 7). One of the pair was processed for immunohistochemical analysis and the other for ELISA analysis. The immunohistochemical analysis was designed to assess the types of cells and adhesion molecules present in the lesional skin. Monoclonal antibodies directed against CD4, CD8, CD1a, HLA-DR, ICAM-1, VCAM-1 and E-selectin were used. During the first week of calcipotriene therapy, no significant changes in the antibody staining pattern or intensity were noted for any of the markers examined, indicating that the cellular expression of the respective marker proteins was unaffected by the treatment. In the ELISA assay, the entire punch biopsy specimen (epidermis and dermis combined) was homogenized for protein extraction. In the lesional skin, interleukin (IL)-10 level increased significantly from day 0 to day 3 with calcipotriene treatment. This elevated level was maintained at day 7. IL-8 level, on the other hand, decreased significantly from baseline to day 3 with the deltanoid, and the reduced level was sustained at day 7. In vehicle-treated psoriatic skin however, no notable changes in IL-10 and IL-8 levels were detected during the first week of therapy. Thus, despite the lack of clinical and immunohistochemical changes early in the treatment, the two cytokine levels were significantly modulated by calcipotriene. Significant changes in IL-10 and IL-8 levels predating the clinical improvement in psoriasis indicate that they are not simply a reflection of improving skin disease. Rather, they are consistent with the possibility that modulations of IL-10 and IL-8 may have contributed to the clinical improvement that followed. The known biological activities of these

cytokines must of course be compatible with this view.

IL-10 is a type2 (T2) cytokine which inhibits antigen-specific activation and proliferation of T cells<sup>11,12</sup>. It can directly suppress IL-2 secretion by CD4 + T cells<sup>13,14</sup> and therefore, IL-10 has been referred to as an immunosuppressive or anti-inflammatory cytokine. The ability of calcipotriene to raise the level of IL-10 in the lesional skin of psoriasis would be clearly beneficial in improving the disease. Consistent with this, direct administration of IL-10 to psoriatic patients has been reported to improve the condition<sup>15</sup>.

Unlike IL-10, IL-8 is a pro-inflammatory cytokine, known to be chemotactic for polymorphonuclear cells<sup>16</sup> and T lymphocytes<sup>17</sup>. In addition, IL-8 promotes proliferation of keratinocytes<sup>18</sup> and endothelial cells (i.e. an angiogenic factor)<sup>19,20</sup>. These properties make IL-8 a clear pro-psoriasis cytokine and indeed, its levels are elevated in lesional skin of psoriasis. A recent demonstration that intravenous administration of antibody directed against IL-8 improves psoriasis<sup>21</sup>, indicates the importance of IL-8 in the maintenance of psoriatic phenotype. Similar to IL-10, IL-8 level was significantly affected early (day 3) in calcipotriene therapy, when clinical changes were not apparent. Significant reduction in its level was maintained at day 7.

Therefore, the early induction of IL-10, an immunosuppressive cytokine, and concomitant reduction of IL-8, pro-inflammatory cytokine, pre-dating the improvement of psoriasis clearly demonstrate that one of the mechanisms whereby calcipotriene improves psoriasis affect cytokine modulation. Cell types responsible for the elaboration of the cytokines, however, were not examined in this study.

More recently, the ability of deltanoids to directly affect T cells in psoriasis was studied utilizing the severe combined immunodeficient (SCID) mice. In this model, human skin is grafted onto the immunodeficient mouse. By virtue of their immunodeficiency, the SCID mouse is unable to reject grafted human skin and its viability is preserved<sup>22</sup>. When nonlesional (symptomless) skin from a psoriatic patient is grafted onto the SCID mice, clinically normal skin appearance is maintained<sup>22,23</sup>. Injection of autologous lymphocytes from the skin donor, activated *ex-vivo*, into the dermis of the

graft (symptomless psoriasis skin) causes the skin to become scaly and thickened like in psoriasis. Histologically, the changes are consistent with involved skin of psoriasis with parakeratosis, elongated rete pegs and dermal neo-angiogenesis<sup>23</sup>. In this model system, when the patients' lymphocytes are treated with CSA prior to injection, the ability of the T cells to transform symptomless skin to psoriasisiform skin is markedly blocked. Similarly, pretreatment of the T cells with 1,25(OH)<sub>2</sub>D<sub>3</sub> blocks, by a comparable magnitude as CSA, transformation of clinically normal skin into a psoriasisiform phenotype. Since calcipotriene is a synthetic analogue of 1,25(OH)<sub>2</sub>D<sub>3</sub> with equipotent binding affinity to the vitamin D receptor, it is likely to have a similar effect in this model system.

Clearly, the ability of deltanoids to inhibit keratinocytes proliferation and promote their differentiation is completely compatible with their antipsoriatic action. However, there now is solid evidence that they also affect the immune system, like other established antipsoriatic medications, to bring about the desired clinical improvement.

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