Immunomodulation and Safety of Topical Calcineurin Inhibitors for the Treatment of Atopic Dermatitis

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Abstract
Atopic dermatitis (AD) is a chronic or chronically relapsing inflammatory skin condition that primarily affects children. Topical corticosteroids have been the mainstay of treatment since the late 1950s. While providing excellent short-term efficacy, topical corticosteroid usage is limited by potential adverse effects, including impairment of the function and viability of Langerhans cells/dendritic cells. The recently introduced topical calcineurin inhibitors pimecrolimus cream 1% (Elidel\textsuperscript{\textregistered}) and tacrolimus ointment 0.03 and 0.1% (Protopic\textsuperscript{\textregistered}) exhibit a more selective mechanism of action and do not affect Langerhans cells/dendritic cells. For the immune system of young children ‘learning’ to mount a balanced Th1/Th2 response, this selective effect has particular benefits. In clinical experience, topical calcineurin inhibitors have been shown to be a safe and effective alternative to topical corticosteroids in almost 7 million patients (>5 million on pimecrolimus; >1.7 million on tacrolimus). Topical pimecrolimus is primarily used in children with mild and moderate AD, whereas tacrolimus is used preferentially in more severe cases. None of the topical calcineurin inhibitors have been associated with systemic immunosuppression-related malignancies known to occur following long-term sustained systemic immunosuppression with oral immunosuppressants (e.g., tacrolimus, cyclosporine A, and corticosteroids) in transplant patients. Preclinical and clinical data suggest a greater skin selectivity and larger safety margin for topical pimecrolimus.

Introduction
Atopic dermatitis (AD) is one of the most common childhood skin diseases, affecting up to 25% of the population in the United States, Europe, and Japan. The onset of AD is in the first year of life in 60% of affected individuals, and before the age of 5 in at least 85% [1, 2]. Similar to other atopic diseases (e.g., asthma and allergic rhinitis), there has been a dramatic 2- to 3-fold increase in the incidence of AD over the last 30 years in industrialized countries. However, in countries with principally rural or agricultural areas, the prevalence has remained much lower [3]. Recent studies have shown clearing of...
AD around puberty in approximately 50% of affected patients [4, 5]. Therefore, the lifetime prevalence of AD in children is 10–20%, and the lifetime prevalence in adults is 1–3% in industrialized countries [3]. AD also has dramatic effects on patient quality of life, leading to distress, anxiety, sleep disturbance, poor self-esteem and low self-confidence. In fact, 60% of the daily activities of school-age children are adversely affected [6–9].

### Atopic Disease and Dysregulation of the Immune System

Although the cause of AD is not well understood, it is often associated with the development of other allergic diseases such as asthma and allergic rhinitis [2]. In a prospective birth cohort study in infants with an atopic family history and AD at the age of 3 months, the risk of developing asthma at age 5 was shown to be over 50% [10]. In another study by Castro-Rodriguez et al. [11] in children with AD and parental asthma, 75% of the children developed asthma at school age. Severity of AD seems to have a major effect on the risk of developing respiratory allergies, and more than doubles the chances of asthma or allergic rhinitis as opposed to children with mild AD [2, 12]. These studies indicate that the course toward an allergic career is determined early in life.

AD appears to be caused by a complex interaction of genetic and environmental factors that result in the dysregulation of the immune system [3, 13]. The pattern of immune dysregulation is different at early stages of an atopic skin inflammation compared with a chronic AD lesion [14, 15], with a dominant Th2 pattern (IL-4, IL-5, IL-13) in acute AD, as seen in young infants. Interestingly, the Th2-dominant immune response seen in early AD lesions or in young children with AD is qualitatively similar to the pattern observed in the immune response in young, non-atopic children: Th2 dominance, impairment of Th1. It could be speculated that this is one of the factors making young infants particularly susceptible to AD.

#### Th2/Th1 Imbalance in Newborns and Young Infants

In newborns and young infants, T cell function is characterized by a poorly sustained Th1 (IFN-γ) response and a propensity toward Th2 (IL-4, IL-13) production [16]. IFN-γ production in response to polyclonal stimuli is diminished at birth, but increases thereafter through 3 years of age, when it approximates adult levels [17]. The impaired Th1 may be related to a lower secretion by antigen-presenting dendritic cells (APCs) of the Th1 polarizing cytokine IL-12 in response to Toll-like receptor ligands. Although neonatal T cells are somewhat limited in their ability to produce the Th2 cytokine IL-4, the production of IL-4 starts to rise rapidly at 4 months of age and peaks at higher-than-adult levels late in infancy before eventually declining to adult levels [16]. This results in a Th2/Th1 imbalance with predominance of Th2 over Th1 responses in older infants and children.

Dendritic cells (DCs) are the most potent APCs. Relatively early in gestation, DCs begin seeding peripheral tissues, and DC networks are readily recognizable in various tissues of the neonate. However, the density of DCs in these networks is lower than in adult tissue, and these DCs express lower levels of major histocompatibility complex (MHC) class II molecules, suggesting a decreased ability to elicit T cell activation. In many tissues, but particularly in the lung, DCs develop slowly with age and are impaired in neonates in their ability to respond to activation stimuli by increasing surface expression of MHC class II molecules and co-stimulatory molecules [18–20]. Langerhans cells (LCs) are DCs found in non-lesional and lesional skin and play a key role in the activation of T cells promoting the switch from naïve to effector cells in the lymph nodes. In contrast, inflammatory DCs have been reported to be highly expressed in lesional skin, but not in non-lesional skin [15, 21].

While the inability of the neonatal immune system to mount a strong cell-mediated immune response (Th1) may not result in clinical pathology in most cases, any external factors further impairing the maturation of a Th1 response may aggravate the innate defect to a level of a manifest clinical disease. Given the important role of APCs (especially LCs/DCs in the skin and lymph nodes) in enabling and maintaining a balanced Th2/Th1 response, a decreased function of this cell type would adversely affect the maturation of the immune response (fig. 1).

#### Topical Treatments for AD

In 1953, Sulzberger et al. [22] introduced the first topical corticosteroid (hydrocortisone) into clinical practice, providing a breakthrough in the treatment of atopic eczema. Since then, modifications of the basic molecular structure of hydrocortisone have led to the development of more potent topical steroids with the benefit of increased efficacy but the disadvantage of increased potential side effects. These side effects include skin atrophy,
striae, telangiectasia, acneiform eruptions, and the risk of absorption, leading to systemic effects such as hypothalamic-pituitary-adrenal (HPA) axis suppression. The mechanism of action of topical corticosteroids involves binding to an intracellular receptor, which forms a complex that interacts with DNA and affects transcription of a number of genes in a wide variety of cells [23]. On a molecular level, corticosteroids block the synthesis of inflammatory cytokines by affecting the nuclear factor kappa B pathway. On a cellular level, corticosteroids, even applied topically, affect the function and viability of LCs. Topical short-term treatment of even low potency corticosteroids eliminates LCs in the skin by inducing apoptosis. Experimental data show that after stopping application of topical corticosteroids, repopulation of the epidermis with LCs takes weeks [24–27].

Tacrolimus ointment, available in the United States since 2001, has been developed as a topical formulation of a systemic immunosuppressant used widely in transplantation (Prograf®, Fujisawa Healthcare, Inc., Deerfield, Ill., USA). There is ample documented experience with the oral formulation of tacrolimus demonstrating its effectiveness as a systemic immunosuppressant and its risk associated with systemic immunosuppression, namely, the risk of immunosuppression-related lymphoproliferative diseases [e.g., Epstein-Barr virus (EBV), positive B cell lymphomas] [28, 29]. Topically applied tacrolimus ointment at concentrations of 0.03 or 0.1% has been shown to be effective in the treatment of AD and is approved for the treatment of moderate to severe AD [30–34].

Pimecrolimus cream 1% was the first calcineurin inhibitor developed specifically for the treatment of AD, it was launched in the United States in 2002 with an approval for the treatment of mild to moderate AD. Short- and long-term trials including pediatric and adult patients with AD have demonstrated the effectiveness of pimecrolimus [35–44].

On a molecular level, both tacrolimus and pimecrolimus selectively inhibit the activation of T cells by inhibiting calcineurin, an enzyme required for dephosphorylation of the inactive cytosolic form of the T cell transcriptional regulatory factor known as nuclear factor of activated T cells [45, 46]. The inactive form of nuclear factor of activated T cells cannot enter the nucleus, so the production and release of inflammatory cytokines as well as T cell proliferation is inhibited. This was demonstrated in a study using T cell clones isolated from the skin of AD patients [45]. Pimecrolimus potently inhibited the proliferation of antigen-specific stimulated T cells and inhibited the production and release of inflammatory cytokines characteristic of both Th1 and Th2 cells [45, 47]. Furthermore, it was shown that pimecrolimus inhibits the expression of the cell surface co-receptor CD134. CD134 expression is increased in activated T cells and acts by inhibiting apoptosis of these cells. By suppressing CD134, pimecrolimus inhibits the survival of antigen-activated T cells [45, 47].

On a cellular level, recent studies demonstrated that while both pimecrolimus and corticosteroids inhibit T cell activation [25, 48], only pimecrolimus is selective, as it does not suppress viability and function of LCs in animals (fig. 2) [26, 49] and in humans (fig. 3) [25]. More specifically, in an in vitro study of mononuclear cells obtained from healthy volunteers, corticosteroids have been shown to cause apoptosis of monocyte-derived DC (M-DC) precursors and inhibit the expression of DC-specific antigens such as CD1a, CD40, and CD80. In addition, corticosteroids inhibit the expression of critical DC factors such as CD83 and CD86, the synthesis of IL-12p70, and the ability of DCs to activate primary CD4+ T cell proliferation [24]. Topical corticosteroids also have an adverse effect on IL-12 expression in LCs/DCs. IL-12
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is a major Th1-promoting cytokine and, therefore, a lack of IL-12 may further delay the maturation of the newborn’s immune system [50]. It should be noted, that, while tacrolimus was not tested in most of the experiments above, it is likely that the effects observed are characteristic of the topical calcineurin inhibitor class. The effect of corticosteroids on DCs seems to be more pronounced in young children than in adults, as suggested in a study by Mainali et al. [51]. A comparison of the effect of dexamethasone on M-DCs obtained from cord blood with M-DCs obtained from adult blood demonstrated that dexamethasone sustains and enhances endocytotic activity, increases the expression of Th2 cytokines, and reduces Th1-promoting IL-12 in cord blood M-DCs, but

Fig. 2. Effect of pimecrolimus and corticosteroids on LC density in murine epidermis. After twice-daily application for 5 days of pimecrolimus or corticosteroids, LC density was assessed. In contrast to pimecrolimus, topical corticosteroids significantly reduced LC density in murine epidermis. Reproduced with permission from Meingassner et al. [49].

Fig. 3. Effect of pimecrolimus and corticosteroids on LC and T cell densities in human skin with AD. a Betamethasone valerate (BMV) cream, a mid potent topical corticosteroid, significantly reduced the number of LCs, whereas pimecrolimus, a topical calcineurin inhibitor, had no effect, being comparable to control. b In contrast, BMV and pimecrolimus both reduced the number of T cells. * p < 0.01 vs. control. Reproduced with permission from Hoetzenecker et al. [25].
has only marginal effects on adult blood M-DCs. Taken together, the results demonstrate that topical calcineurin inhibitors act more selectively on the skin immune system and do not induce apoptosis in LCs/DCs. Therefore, these agents provide an interesting innovation in the treatment of AD and raise questions about the use of corticosteroids in young children with a developing immune system (fig. 1).

**Systemic Absorption of Topical Treatments for AD**

In a pharmacokinetic study in children treated with hydrocortisone cream 1% (no occlusion), an increase of cortisol plasma levels over baseline of 77–967 ng/ml (212–2,669 nmol/l) has been reported, with HPA axis suppression observed in 38% of these patients [52]. In another study of hydrocortisone cream 1% in adults with widespread AD (at least 50% BSA affected), plasma cortisol levels increased over baseline to up to 889 nmol/l [53]. These data indicate the potential for significant absorption of even low-potency topical corticosteroids in patients with AD.

More recently developed topical corticosteroids seem to be associated with lower plasma levels. In a study of fluticasone propionate cream 0.05%, plasma levels of fluticasone ranged between 0.07 and 0.39 ng/ml. Although the medication was applied under occlusion, thus enhancing skin penetration, this effect was countered by the fact that the study was conducted in healthy volunteers where an intact skin barrier function may limit absorption [54]. Because the biological effect of fluticasone propionate is higher than the equimolar amount of hydrocortisone acetate, it is not surprising that some cases of HPA axis suppression were reported with fluticasone propionate cream 0.05%. In a study in children as young as 3 months with moderate to severe and extensive AD (64% BSA treated at baseline), only 2 children of the 43 enrolled exhibited HPA axis suppression [55]. Using more potent topical corticosteroids, HPA axis suppression is achieved in most patients, as reported for clobetasol propionate lotion 0.05%, where 55% of AD patients had measurable HPA axis suppression after 2 weeks [56]. An expert discussion on the risk of HPA axis suppression with the United States Food and Drug Administration (FDA) (FDA–AC, March 24, 2005) noted that the level of sensitivity of the current HPA axis tests is only 70% (leaving 30% of cases undiagnosed), and that an undiagnosed HPA axis suppression carries the risk of ‘sudden death’. The risk, however, is extremely low and is clinically acceptable as long as topical corticosteroids are used under supervision of a physician [57].

Due to its high lipophilicity, pimecrolimus penetrates into the skin, but its absorption through the skin into the systemic circulation is very low, lower than tacrolimus and much lower than steroids [58]. For rat, pig, and human skin, pimecrolimus demonstrated a 9- to 10-fold lower permeation through skin in vitro than tacrolimus when comparing identical solution of both compounds. Similar results were obtained using the marketed Elidel® cream (Novartis Pharmaceuticals Corporation, East Hanover, N.J., USA) and Protopic® ointments (Fujisawa Healthcare) [59]. The permeation rates through human skin measured in vitro were found to be lower with pimecrolimus than with tacrolimus, with pimecrolimus permeation rates lower than tacrolimus 0.1% ointment by a factor of about 6.

When pimecrolimus cream 1% is applied to the skin, very little enters the systemic circulation (fig. 4) [60]. The systemic absorption of pimecrolimus cream 1% has been extensively evaluated in children and adults with moderate to severe AD [38, 61–63]. In pharmacokinetic studies of children (75 patients and 366 sam-

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**Fig. 4.** Permeation (penetration) of pimecrolimus cream 1%. When topically applied, only small amounts of pimecrolimus reach the dermis [60]. Data obtained from minipig skin studies using radioactive labeled pimecrolimus.
In most patients in whom blood concentrations of pimecrolimus have been measured, the concentration of pimecrolimus was too low to allow the calculation of the area under the concentration-time curve (AUC) or the AUC sampling scheme was not adequate [64]. In 8 of 20 pediatric patients (2–14 years of age) with a suitable blood sampling scheme, the AUC([0–24 h]) ranged from 10.8 to 37.6 ng·h/ml. The mean AUC in children (including infants) was 22.5 ng·h/ml. In adults, the AUC([0–24 h]) ranged from 5.0 to 22.8 ng·h/ml, with a mean AUC([0–24 h]) of 11.7 ng·h/ml [only 10 of 30 patients had measurable AUC([0–24 h])] [65].

In a study by Harper et al. [68], the pharmacokinetics of tacrolimus were studied in 39 pediatric patients (aged 6–12 years) with moderate to severe AD, affecting 11–94% BSA. The systemic exposure of tacrolimus tended to increase as a larger affected BSA was treated: on day 1 of treatment for patients with affected BSA ≤1,500 cm², the mean AUC([0–24 h]) was 5.2 ng·h/ml; for affected BSA >1,500 and ≤3,000 cm², the mean AUC([0–24 h]) was 17.5 ng·h/ml, and for affected BSA >3,000 and ≤5,000 cm², the mean AUC([0–24 h]) was 11.0 ng·h/ml. The corresponding values on day 14 were 3.3, 15.4 and 11.4 ng·h/ml.

The skin lesions in patients with Netherton syndrome represent a therapeutic challenge as the skin barrier function in these patients is significantly impaired, thus increasing absorption of any topically applied treatment. Tacrolimus ointment 0.1%, when used in children with Netherton syndrome, induced blood levels (up to 37 ng/ml) well within or even above the range of those observed with orally administered Prograf for transplantation [69], establishing the potential for systemic immunosuppression with tacrolimus ointment in children with severely impaired skin barrier function. In contrast, pimecrolimus cream used in Netherton syndrome (99% BSA treated) demonstrated blood levels below 2.4 ng/ml over the whole treatment period [70].

**Tacrolimus versus Pimecrolimus in Transplant Models**

A number of transplantation models in animals are available to assess the efficacy of a drug to provide systemic immunosuppression. In one model, a rat model of graft-versus-host reaction [71–73], injecting MHC-mismatched spleen cells into the hind foot pad of the rat caused a graft-versus-host reaction and lymph node enlargement near the site of injection. The doses (subcutaneous application) required to inhibit 50% swelling were 66 times greater for pimecrolimus (20 mg/kg) than for tacrolimus (0.3 mg/kg) and 8 times greater than for cyclosporine A (CyA; 2.5 mg/kg).

In another rat model of immunosuppression, pimecrolimus was found to be 48 times less effective than tacrolimus (subcutaneous application) for inhibiting the production of IgM anti-sheep antibodies when rats were injected with sheep red blood cells [71, 73]. Furthermore, in a rat model of allogeneic kidney transplantation, the oral pimecrolimus dose required for 100-day survival was 15.6 mg/kg compared with only 5.0 mg/kg of CyA and 1 mg/kg of tacrolimus [71–73]. The ability to achieve systemic immunosuppression in this model was 3 and 15 times less for oral pimecrolimus than for CyA and tacrolimus, respectively. These experiments in animals demonstrate that systemic exposure to pimecrolimus (following oral or subcutaneous application) is significantly less effective in suppressing the systemic immune system than other calcineurin inhibitors. Consistent with these findings, tacrolimus and CyA, but not pimecrolimus, provide sufficient immunosuppression to be used clinically to prevent organ rejection/grant-versus-host disease after transplantation.

In contrast to the above-described studies in models of systemic immunosuppression, oral pimecrolimus was shown to be as effective as tacrolimus in inhibiting skin inflammation in mice and rats. Interestingly, in these experiments, the draining lymph nodes were not affected regarding primary immune response, weight, number, and functionality of lymph node cells, as was seen with tacrolimus [74].
Carcinogenicity Studies with Pimecrolimus and Tacrolimus

As with all recently developed treatments, pimecrolimus and tacrolimus have been tested in numerous animal studies designed specifically to further explore the toxicity of the molecule and increase drug exposure to observe toxic effects. In dermal toxicology studies in rodents, pimecrolimus dissolved in an ethanolic solution (enhancing penetration through the skin) was applied to mice topically, administered continuously over 104 weeks (the life span of a mouse), resulting in a mean daily exposure [AUC (0–24 h)] of 1,040 ng·h/ml. No drug-related malignancies were observed, establishing a non-observed adverse event level (NOAEL) in rodents (fig. 5a) [64, 65, 75]. In a similar study in mice using a higher dose achieving an AUC (0–24 h) of 1,770 ng·h/ml, lymphoproliferative changes (including lymphoma) were observed after 13 weeks [64]. To further explore the toxicity profile of the molecule in an aim to develop an oral formulation of pimecrolimus for the treatment of psoriasis, the compound was administered orally in cynomolgus monkeys. At a dose of 15 mg/kg/day, animals reached an average systemic exposure of ~1,200 ng·h/ml. After 39 weeks, 1 of 8 monkeys developed lymphoproliferative disease, indicating that oral administration at a dose of 15 mg/kg/day approaches a threshold for systemic immunosuppression, thus allowing the development of lymphoproliferative disorders [65, 75]. Similarly, tacrolimus doses of 10 mg/kg/day resulted in lymphoproliferative changes in certain cynomolgus monkeys after 90 days [76]. The results in monkeys confirm the potential observed previously in rodents for producing lymphoproliferative disease upon sustained and prolonged exposure to high doses well above 25 times the average exposure at the maximally recommended human dose (referred to as MRHD), after topical pimecrolimus cream 1%.

Dermal toxicology studies with tacrolimus ointment indicate a NOAEL at an AUC (0–24 h) of 189 ng·h/ml, while lymphomas were noted in mice at 530 ng·h/ml, suggesting a threshold level at AUC (0–24 h) above 200 ng·h/ml (fig. 5b) [66, 77, 78].

In order to compare the toxicity profiles of tacrolimus and pimecrolimus, a standardized reference using the mean AUC (0–24 h) of all available AUCs from human pharmacokinetics studies can be established. In adults, the MRHD [AUC (0–24 h)] of tacrolimus is 20.4 ng·h/ml [77] in a particular study, and 23 ng·h/ml for pimecrolimus. Compared to this baseline, the NOAEL for tacrolimus established in dermal toxicity studies is ~10 times the MRHD [AUC (0–24 h)] and for pimecrolimus it is ~45 times the MRHD. The lowest observed adverse event levels (LOAELs) established in the same model are ~26 times the MRHD for tacrolimus and ~77 times the MRHD for pimecrolimus (see fig. 5). (In cynomolgus monkeys, the LOAEL for oral pimecrolimus is ~52 times the MRHD).

Toxicology data suggest an increased safety margin for pimecrolimus compared with tacrolimus, which may become clinically relevant in cases of extensive and/or prolonged use of a topical calcineurin inhibitor, especially in children.
Clinical Experience with Topical Calcineurin Inhibitors

Pimecrolimus Cream

In clinical studies, approximately 20,000 patients have been treated with pimecrolimus cream 1%, including nearly 3,000 infants and over 7,000 children [65]. In clinical practice, over 5 million patients have been treated, of which nearly 2 million are below the age of 5 years. The average patient in clinical practice is treated intermittently for approximately 45 days per year and uses less than 2 g of pimecrolimus cream 1% per day [65].

Pimecrolimus cream 1% has been studied in short- and long-term vehicle-controlled trials in infants, children, and adults. In 5 short-term studies, pimecrolimus cream 1% applied twice daily for 3–6 weeks was effective at controlling the signs and symptoms of AD in patients with predominantly moderate disease, using the Atopic Dermatitis Severity Index, Investigator Global Assessment, or Eczema Area and Severity Index (EASI) severity scores as endpoints [25, 79–82]. In long-term studies of up to 1 year, pimecrolimus cream 1% applied twice daily as needed reduced AD flares and thus reduced the need for topical corticosteroids [35, 37, 79, 83].

Tacrolimus Ointment

In clinical studies, including post-marketing studies, more than 20,000 patients have been treated with tacrolimus ointment 0.03 and 0.1%. Of those, more than 7,500 were pediatric patients. Over 1.7 million patients have been treated with tacrolimus ointment in clinical practice since 2001 [78].

In several short-term studies, tacrolimus ointment has been shown to be an effective treatment for AD in adults and children [30, 31, 33, 34, 84]. Patients treated with tacrolimus ointment, 0.03 and 0.1% applied twice daily for up to 6 weeks showed improvement in outcomes such as modified EASI and Investigator Global AD Assessment. In a 1-year study, tacrolimus ointment 0.1% applied twice daily to areas of actively diseased skin was shown to be effective as assessed by modified EASI and Investigator Global Assessment, with ointment use decreasing over time [32].

Lack of Evidence of Systemic Immunosuppression with Pimecrolimus Cream

In a 2-year study, the use of pimecrolimus cream 1% was evaluated to determine if there were any effects on the ability of children to mount an immune response after vaccination [85]. Ninety-one patients who had been treated with pimecrolimus for up to 2 years were vaccinated against diphtheria, measles, rubella, and tetanus. The mean BSA involved was 27.6% at baseline, with a median number of days of application of 377.5. There were no differences in seropositivity for these vaccines among those treated or untreated with pimecrolimus cream 1% (fig. 6) [85]. These results demonstrate that pimecrolimus cream 1% does not interfere with the development of immune responses to vaccines. This has also been shown to be true for tacrolimus ointment 0.03% in a similar study of pneumococcal seroconversion. The 23 observed patients had moderate to severe AD, with a mean BSA affected of 28.1%, and 91% had a greater than 4-fold increase in titer for at least 4 of 12 pneumococcal serotypes [86].

Cell-mediated, delayed-type hypersensitivity reaction against a range of antigens is a common method of assessing immunocompetence. Delayed-type hypersensitivity was used to test immune function in one study of pimecrolimus cream 1% with a total of 112 subjects who completed 1 year of treatment with pimecrolimus cream 1% (82 pimecrolimus cream 1%; 30 vehicle), and one study of tacrolimus ointment 0.1% with 56 subjects evaluated after 6 months and 15 subjects evaluated after 1 year [32, 37]. The results of both studies suggest that use of pimecrolimus cream 1% or tacrolimus 0.1% has no effect on the
ability to develop a normal T cell-mediated immune response.

In a study that included 76 children who received pimecrolimus for 2 years, pimecrolimus cream 1% was effective for reducing the EASI by a mean of 70.8% from baseline [40]. In addition, pimecrolimus 1% was safe and well tolerated in these children. Compared with the first year of the study, common childhood infections declined over time and throughout the second year of treatment with pimecrolimus cream 1%. The decreased rate of infections after long-term treatment in this study suggests that pimecrolimus cream 1% does not have an adverse effect on the systemic immune system over time. With respect to malignancies, significantly less were reported in patients using pimecrolimus (1 case per 9,500 patients) compared with patients treated with topical corticosteroids or placebo (1 case per 800 patients). The malignancies reported in patients using pimecrolimus in clinical trials were 1 case of colon cancer and 1 case of squamous cell carcinoma, both in patients above 65 years of age (fig. 7) [60, 65].

**Experience with Topical Calcineurin Inhibitors in Clinical Practice: Spontaneous Reports of Adverse Events**

The FDA collects spontaneous reports of drug-related adverse events on a regular basis, and those reported for topical calcineurin inhibitors were presented in September and October of 2004 by the Office of Drug Safety [87, 88]. While no immunosuppression-related malignancies (see below) attributable to the use of topical calcineurin inhibitors were reported, a case report of sepsis in a child with severe AD who had used tacrolimus ointment 0.1% in large amounts was pointed out. Although sepsis in a child with severe AD is possibly related to the underlying disease, the detection of a blood level of tacrolimus of 3.5 ng/ml, measured 2 weeks after stopping administration of tacrolimus, is noteworthy.

**Lymphoproliferative Disease in Patients with Systemic Immunosuppression: A Different Clinical Entity in Humans**

Three types of conditions have been associated with immunosuppression-related lymphoproliferative diseases in humans: (1) congenital immunodeficiencies; (2) acquired immunodeficiencies, and (3) post-transplantation immunodeficiency/suppression.

**Congenital Immunodeficiencies**

Virtually all congenital immunodeficiency syndromes that affect T cells confer an increased risk of lymphoproliferative disease [89–91]. This risk may be considerable, rising to approximately 10% in patients with Wiskott-Aldrich syndrome and up to 30% in patients with X-linked lymphoproliferative disease. Patients with the more frequently observed diagnosis of common variable immunodeficiency have been reported to have a 23- to 100-fold increase in risk of malignant lymphoma [89, 91, 92]. Sig-
nificant proportions of these immunodeficient patients may have eczema or chronic dermatitis as part of the underlying disease [93]. Management of the eczema frequently antedates final diagnosis, and immunodeficiency diseases may go undiagnosed for long periods of time (such as in the case of common variable immunodeficiency). Thus, consideration of underlying immunodeficiency and predisposition to lymphoma should be considered when lymphoproliferative disorders develop in individuals, particularly children, with severe or refractory eczema.

**Acquired Immunodeficiencies**

Acquired immunodeficiencies are all associated with an increased risk of the development of lymphoproliferative disease, usually in the context of immune suppression with some secondary stimulus, such as acquisition or reactivation of a viral infection with agents such as HIV, EBV, cytomegalovirus, herpes simplex virus 8, or hepatitis virus C [94, 95]. Use of anti-T-cell antibodies in solid organ and hematopoietic stem cell transplantation is also associated with an increased risk of lymphoproliferative disease [96, 97]. While the risk may be conferred by inducing lymphopenia, it is also noteworthy that these agents may exert direct mitogenic effects [98].

**Post-Transplantation Immunodeficiency/Suppression**

The most pertinent data related to the potential relationships of topical immunosuppressants to immunodeficiency-related lymphoproliferative disease (IRLD) emerge from an analysis of individuals with post-transplant lymphoproliferative disease (PTLD). The incidence of lymphomas after hematopoietic stem cell transplantation is very low (0–0.7%) [94] in patients (pediatric and adult) who are related and HLA-matched, despite aggressive immunosuppressive conditioning regimens containing agents such as cyclophosphamide, fludarabine, and total body irradiation as well as post-hematopoietic stem cell transplantation systemic administration of calcineurin inhibitors, methotrexate, mycophenolate, and corticosteroids. The incidence rises substantially when the donor T cells are removed (especially in the absence of concomitant B cell depletion) and with increasing HLA disparity. For example, the relative risk of early onset PTLD appears to be 4-fold greater with HLA disparity and 13-fold greater with T cell depletion of stem cells [99]. PTLD almost uniformly occurs within the first 8 months, with a median time of 3 months. This contrasts with the timing in solid organ transplantation recipients, who can develop lymphomas early (4–8 months) or late (>1 year) [94].

In contrast, the reported incidence of PTLD in solid organ transplantation patients ranges from 2 to 60% and is dependent on the type of organ transplanted. Generally, there are more cases of PTLD with small bowel transplants than with heart, lung, or liver transplants. Kidney transplantation patients are least affected [94]. It is hypothesized that small bowel transplants have the highest rate of PTLD (approximately 30% in pediatric patients) due to the high concentration of lymphocytes in this tissue and the likelihood of potential transfer of EBV-infected donor lymphocytes to the often seronegative pediatric patient. Regardless of organ type, there is a general relationship between the intensity of immunosuppression and the risk of PTLD. The majority of PTLD described has been B cell phenotype, with a reported rate of T cell disease of only approximately 10%, even with very long follow-ups [100, 101].

The concern that topical calcineurin inhibitors would cause lymphoproliferation can be addressed best with what we understand to date about IRLD. This information has been accumulated over many years of observing patients with acquired and congenital immunodeficiency. In fact it is reassuring, rather than disturbing, that the cases of lymphoma observed in primate animal studies were histologically consistent with a virus-related lymphoproliferative state after prolonged, high-dose immunosuppression [65]. Thus, if these lesions represent IRLD associated with pimecrolimus, then the mechanism and results fit the model of IRLD found with other high-dose immunosuppressants in humans.

However, current topical pimecrolimus use in children and adults, which consists of intermittent use of a poorly absorbed drug, is not consistent with IRLD models. First, neither the complexity nor the intensity of the topical immunosuppression observed with pimecrolimus cream 1% is sufficient to be plausibly related to IRLD. Even if intermittent detectable systemic levels are occasionally achieved in some patients [38], this does not represent a risk in humans, based on the experience with sustained high systemic levels of the calcineurin inhibitor CyA in patients with aplastic anemia (i.e., patients who, like those with AD, do not suffer from any of the other complications associated with a transplant). In these patients, systemic exposure to CyA alone is insufficient to give rise to increased lymphoma risk [102, 103]. This observation can also be extrapolated to negate the argument that draining lymph nodes might have higher levels of pimecrolimus than the skin; it is implausible that levels in the lymph nodes of topically treated patients would be greater than that found in patients with rheumatologic
diseases or aplastic anemia who are on daily oral calcineurin inhibitors and in whom the therapeutic levels are routinely maintained for months or even years.

Second, the association of EBV with all types of IRLD is well established [89], and there may have been a viral association in the non-human primate case of lymphoma [75]. No such association with EBV has been reported for topical calcineurin inhibitor-treated cases to date. It is likely that there would be an association with EBV if these were indeed cases of IRLD.

Third, the range of histologic appearance (and progression from atypia to frank lymphoma) of IRLD lesions in humans has been characterized and reported by many groups [94]. No such range or progression is evident in the reported topical calcineurin inhibitor-treated cases [88]. It is implausible that in this group of patients all lesions would have been diagnosed in their most advanced state. In the IRLD literature, a preponderance of B cell disease is reported while T cell disease is rare [90]. This is the opposite of what has been seen in the spontaneous cases reported to date. If T cell PTLD occurs, it generally does so after 8–10 years of continued immunosuppression [94, 95], not after the relatively short time that these patients were treated. The literature discloses that T cell PTLD is aggressive and does poorly under treatment. All patients with lymphoma who use pimecrolimus cream 1% that have been reported to date appear to be responsive to therapy and are doing well. If these reports indeed were representative of pimecrolimus-induced IRLD, it would be difficult to explain why virtually every signature characteristic of IRLD described in the literature fails to be echoed in them.

Finally, it is important to recognize that patients with PTLD have had meticulous pre-transplant as well as careful and frequent post-transplant assessments. Thus they are known to be lymphoma-free at transplant, their clinical course and drug utilization is well understood, and, therefore, attribution of lymphoma to immunosuppression associated with transplant is likely correct. In contrast, some of the reported cases of putative pimecrolimus- or tacrolimus-related IRLD are very suggestive of underlying disorders (e.g., peripheral T cell lymphoma) mistaken for AD, with the correct diagnosis being made only after treatment had been initiated. Careful assessment of pre-existing lymphoma risk (particularly in children who may have congenital immunodeficiency and associated eczema) and evaluation of adults for pre-existing syndromes such as cutaneous T cell lymphoma and mycosis fungoides is therefore essential if attribution and understanding of risk is to be accurately ascertained.

**Summary**

AD is a prevalent disease in young children that can have a profound impact on physical and social well-being, and is often the first step in the atopic march. The impaired Th1 response that is seen in atopic children is qualitatively similar to the one seen in healthy infants, but is more pronounced. In order for a mature Th1 response to develop, APC and especially DC function is important. Factors that adversely affect the viability and function of these cells have the potential to delay or suppress the maturation of the immune system in young children, thereby promoting a persistent Th2-dominant response pattern.

Topical corticosteroids, in contrast to topical calcineurin inhibitors, reduce viability and function of LCs/DCs of skin and lymph nodes, contributing to a reduction in Th1-promoting cytokines (e.g., IL-12). These recently acquired immunological insights raise further questions about the use of topical corticosteroids in young children. Alternative topical treatments, such as topical calcineurin inhibitors, act more selectively and may provide advantages over older treatments. Within the class of available topical calcineurin inhibitors, pimecrolimus cream 1% seems to exhibit a larger safety margin than tacrolimus ointment.

In contrast to the experience with topical pimecrolimus cream and tacrolimus ointment, systemically administered tacrolimus and CyA have been shown to induce systemic immunosuppression, thus increasing the risk of malignancies in patients having undergone organ transplantation. However, the degree of systemic immunosuppression required is not attainable with topical calcineurin inhibitors. In over 3 years of usage in more than 5 million patients using pimecrolimus cream, and over 1.7 million patients using tacrolimus ointment, the safety of both of these treatments has been established.

**Conclusions**

Today’s immunology insights and data suggest an advantage of topical calcineurin inhibitors over topical corticosteroids for the use in young children. Data suggest that within the class of topical calcineurin inhibitors, pimecrolimus cream exhibits a larger safety margin than tacrolimus ointment.
References


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Immune Modulation and Safety of Topical Calcineurin Inhibitors


