OBSERVATION

Tacrolimus Ointment in the Treatment of Chronic Cutaneous Graft-vs-Host Disease

A Case Series of 18 Patients

Caroline J. Choi, MD; Paul Nghiem, MD, PhD

Background: Tacrolimus (formerly FK 506) is an immunosuppressive drug that works by inhibiting calcineurin, a calcium-dependent protein phosphatase required for immune function. Tacrolimus has been shown to be effective topically in atopic dermatitis and systemically in psoriasis and graft-vs-host disease (GVHD). However, its efficacy in treating cutaneous GVHD when applied topically has not been reported.

Objective: To assess the therapeutic efficacy of 0.1% tacrolimus ointment on chronic cutaneous GVHD in patients with symptoms refractory to systemic corticosteroid therapy.

Results: Tacrolimus ointment effectively treated pruritus and/or erythema in 13 (72%) of 18 patients with chronic GVHD. Responding patients had a rapid effect within several hours to days. Effectiveness was measured by means of patient report, results of physical examination, side-by-side comparisons of tacrolimus vs a vehicle control, and temporal flares of the cutaneous symptoms of the disease in the context of stopping tacrolimus ointment therapy. Because of the progression of GVHD and in 2 cases, loss of drug efficacy, all patients eventually went on to receive more aggressive treatment, including increases in steroid dosage, psoralen–UV-A therapy, and extracorporeal photopheresis.

Conclusions: This case series suggests that tacrolimus ointment has efficacy in treating the erythema and pruritus of steroid-refractory, chronic cutaneous GVHD in most patients. The rapid response of tacrolimus ointment may provide a useful therapeutic bridge to systemic therapies that have slower onset, such as psoralen–UV-A therapy or extracorporeal photopheresis.

Arch Dermatol. 2001;137:1202-1206

From the Department of Dermatology, Brigham and Women's Hospital and Dana Farber Cancer Institute, Harvard Medical School, Boston, Mass (Drs Choi and Nghiem); and the Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Mass (Dr Nghiem).
The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

DESPITE advances in prophylactic treatment and T-cell depletion protocols, chronic graft-vs-host disease (GVHD) remains a frequent complication of allogeneic bone marrow transplantation (BMT), affecting 50% of patients with long-term transplant survival,[1] with a 20% to 40% mortality rate.[2] First described in 1966 by Billingham,[3] the pathophysiology of GVHD involves damage to the host tissue by the pre-BMT conditioning regimen and consequent cytokine release, activation, and clonal expansion of the donor's effector T cells and T- and NK-cell–mediated cytotoxic effects.[4-6] Principal targets of GVHD include the skin, gastrointestinal tract, and liver. The most common manifestations are cutaneous, causing significant morbidity and mortality. Acute cutaneous GVHD symptoms can range from a mild, erythematous morbilliform eruption to a more severe epidermal necrolysis, occurring within 100 days after BMT. This can progress to chronic GVHD, which can be lichenoid or sclerodermatous, with waxing and waning flares of erythema, pruritus, and scaling. Chronic GVHD can also arise spontaneously without previous acute GVHD. Given the broadening indications for BMT and protocols allowing for a greater degree of donor HLA antigen mismatch, treatment of the cutaneous manifestations of GVHD will become an increasingly frequent challenge.

Conventional treatment of chronic GVHD has required prolonged periods of systemic immunosuppressive therapy with potent drugs such as corticosteroids and cyclosporine.[7] These drugs cause significant adverse effects with high morbidity and mortality due to infections and may fail to control the progression of the disease. Topical steroids have been a mainstay of local treatment of cutaneous GVHD, but their use has been limited by adverse effects, including skin atrophy, telangiectasia, and striae cutis distensae. Therefore, alternative approaches to the management of chronic GVHD have been explored to improve the survival and quality of life of patients with GVHD. Psoralen–UV-A (PUVA) therapy and extracorporeal photopheresis (ECP) have been used

PATIENTS AND METHODS

All patients in this case series were seen in the cutaneous oncology clinic at Dana Farber Cancer Institute, Boston, Mass, with the clinical diagnosis of chronic GVHD after allogeneic BMT. All patients were using at least 1 systemic immunosuppressive drug, including corticosteroids, cyclosporine, and mycophenolate mofetil, for control of GVHD. However, their cutaneous symptoms were refractory, and the patients were referred to our clinic for additional therapy for their disease. The clinical diagnosis of GVHD was given to these patients in contrast to other diagnoses of atopic, seborrheic, or contact dermatitis, given the development of cutaneous symptoms shortly after BMT, an incomplete response to high-dose systemic immunosuppression, and the absence of a history of external skin irritants. Patients were excluded from this series if they were concurrently using topical corticosteroids or systemic tacrolimus. The risks and benefits of tacrolimus ointment were discussed with each patient before initiation of therapy.

The mean age of the patients (10 men and 8 women) was 42.8 years (range, 28-59 years). The 18 patients had the following primary diseases: chronic myelogenous leukemia (4 patients), non-Hodgkin lymphoma (4 patients), acute lymphocytic leukemia (3 patients), acute myelogenous leukemia (4 patients), myelodysplastic syndrome (1 patient), chronic lymphocytic leukemia (1 patient), and multiple myeloma (1 patient). Patients had undergone the following types of BMT: matched sibling (6 patients), matched unrelated donor (3 patients), T-cell depletion and matched sibling (5 patients), and T-cell depletion and matched unrelated donor (4 patients).

Because it was not yet available in an ointment form, topical tacrolimus therapy was prepared extemporaneously as a 0.1% ointment as described by Aoyama et al.[25] Tacrolimus powder was compounded with 8% beeswax, 3% cholesterol, and 3% stearyl alcohol, and patients were instructed to apply the ointment 2 to 3 times daily to affected areas. Serum tacrolimus levels were measured to monitor systemic absorption in 2 patients who applied tacrolimus ointment over their entire body and had a response. The clinical course of patients was followed in the weeks and months after the initiation of tacrolimus ointment therapy. Efficacy of the therapy were evaluated by means of subjective patient report and results of physician examination. Evidence of efficacy was also inferred when possible from more objective measures, including side-by-side comparisons of tacrolimus ointment on one side of the body with a petroleum vehicle control ointment on the other, as well as temporal flares of the disease after stopping therapy. Treatment with tacrolimus ointment was discontinued if it appeared that there was no benefit or if the patient experienced adverse effects.

for a variety of T-cell–mediated skin diseases since the1980s,[8,9] and recent case series have suggested that these therapies are also effective for the treatment of chronic GVHD.[10-13] However, because it takes months to see the beneficial effects of PUVA therapy and ECP, and because of the more immediate need to palliate local symptoms of cutaneous GVHD, there has been much interest in developing other topical therapies. Tacrolimus (formerly FK 506) is a superb candidate for the topical treatment of chronic GVHD because of its ability to penetrate the skin, limited profile of adverse effects, and potent immunosuppressive effect.

Tacrolimus is an immunosuppressive antibiotic from the macrolide family, shown to be 10 to 100 times more potent than cyclosporine in the inhibition of T-cell activation.[14-16] This agent works by inhibiting calcineurin, a calcium-activated protein phosphatase, which is necessary for appropriate immune modulation.[17] Tacrolimus has also been shown to inhibit histamine release from mast cells and basophils, which may also contribute to its antipruritic effect.[18,19] Tacrolimus was first used clinically to prevent graft rejection in organ transplantation. It has also been shown to be efficacious in pyoderma gangrenosum, Behçet's disease, and Crohn's disease, and large multicenter studies have reported topical administration to be effective in atopic dermatitis[20,21] and oral administration to be effective in psoriasis.[22] Because tacrolimus does not affect collagen synthesis,[23] there is no risk for skin atrophy, and with topical application, serum levels of the drug remain low or undetectable,[24] thus avoiding the risk for nephrotoxic effects found with oral tacrolimus. The major reported adverse effect of tacrolimus ointment has been a transient burning sensation, making it a safe alternative to topical steroids. We hypothesized that given its immunomodulatory activity and its efficacy in treating other inflammatory cutaneous diseases, tacrolimus ointment would have therapeutic efficacy in treating patients with chronic cutaneous GVHD.

RESULTS

A summary of our results is presented in the **Table**. Eighteen patients were treated with tacrolimus ointment for their refractory chronic cutaneous GVHD. Thirteen patients (72%) responded to the treatment. A response was defined as effective relief of erythema and/or pruritus. Scaling, pain, and "tightness" were also relieved by tacrolimus ointment, as reported by patients. Only 1 patient experienced a negative effect, which was described as an uncomfortable sensation, as he felt that the extemporaneously compounded ointment was "clogging the pores" on his face.

Of 6 patients who performed side-by-side comparisons of tacrolimus ointment vs vehicle control, 4 (67%) had a positive effect of the tacrolimus compared with the control. In addition, 3 patients experienced flares of cutaneous symptoms when stopping application of the tacrolimus ointment for days, with rapid resolution on reapplication of the tacrolimus. Responding patients found that tacrolimus ointment was effective within hours to days. Of the 13 patients with a response, 6 (46%) used it for 3 to 4 weeks; 4 (31%), 2 to 4 months; and 3 (23%), more than 1 year. Two patients who responded to the tacrolimus ointment lost efficacy of the therapy in 10 to 15 weeks. Of the 5 patients who did not respond to tacrolimus ointment, none continued therapy beyond 1

Treatment of Patients With Chronic GVHD*

Patient No./ Sex/Age, y	Effect of Tacrolimus		Side-by-Side Comparison†	Duration of Tacrolimus Therapy	Sites of Chronic GVHD	Sites With Most Benefit	Duration of GVHD Flare Before Tacrolimus Therapy	Primary Disease
	Erythema	Pruritus						
1/F/28	+	++	+	3 mo; followed by tachyphylaxis	Generalized	General	1.5 mo	ALL
2/M/59	+	+	+	3 wk	Generalized	General	5 mo	AML
3/M/49	+	+	+	1 mo	Generalized	General	2 mo	CLL
4/F/49	+	+	+	18 mo	Generalized/face, palms	Face, hands, arms	3.5 mo	CML
5/M/59	++	+	NP	3-4 mo; followed by tachyphylaxis	Generalized	Face, hands, neck	5.5 mo	AML, MDS
6/F/47	+	+	NP	13 mo	Generalized	Face	4 mo	MM
7/M/47	+	+	NP	1 mo	Generalized	General	7 mo	Low-grade NHL
8/F/39	+	+	NP	1 mo	Generalized	General	12 mo	AML
9/M/31	+	+	NP	3 wk	Penis	Penis	6 mo	CML
10/F/48	+	NA	NP	3 mo	Legs, behind knee	Legs	1 mo	NHL
11/M/35	+	NA	NP	12 mo	Lower extremity	Legs	6.5 mo	CML
12/F/29	+	NA	NP	2-3 mo	Hands, feet	Palms and soles	1 mo	MDS
13/M/45	−	+	NP	1 mo	Generalized	Legs	18 mo	NHL
14/F/38	−	−	−	NA	Torso, legs, arms	NA	1 mo	NHL
15/F/44	−	NA	−	1 mo	Generalized	NA	3 wk	CML
16/M/29	−	−	NP	2 wk	Generalized/behind knees	NA	42 mo	ALL
17/M/44	−	−	NP	1 mo	Generalized	NA	8 mo	ALL
18/M/51	−	NA	NP	1 mo	Trunk, lower extremity	NA	24 mo	AML, MDS

*GVHD indicates graft-vs-host disease; plus sign, improved; 2 plus signs, markedly improved; minus sign, no improvement; NA, not applicable; NP, not performed; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; URD, unrelated donor; sib, sibling; PUVA, psoralen–UV-A; and ECP, extracorporeal photopheresis.

†Indicates comparison with petroleum vehicle control ointment.

‡Decadron (Merck & Co, Inc, Whitehouse Station, NJ).

month, and 1 patient described his use of the ointment as "sparing." Serum tacrolimus levels were measured in the 2 patients who were using tacrolimus ointment most intensively and who had responded to an entire-body application of the ointment. During the initial period of heavy application, serum tacrolimus levels were undetectable for one of these patients and 1.7 ng/mL for the other (well below the systemic therapeutic range indicated for the prevention of graft rejection, 5-15 ng/mL).

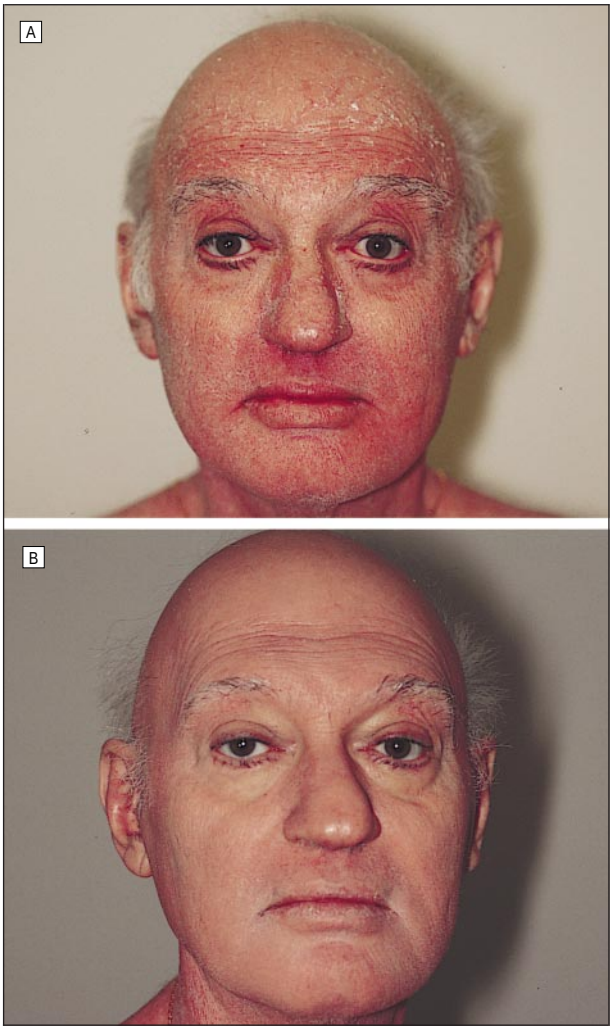
In no patient was tacrolimus ointment alone sufficient to control cutaneous GVHD in an ongoing manner. Four (22%) of 18 patients had long-term improvement of their cutaneous GVHD with adjustment of their oral immunosuppressive therapy, 12 patients (67%) started or are waiting to receive PUVA therapy or ECP, and 5 patients (28%) died secondary to progression of their primary cancer, GVHD, and/or infection.

REPORT OF A CASE

A 59-year-old Egyptian American man (patient 5) received a diagnosis of acute myelogenous leukemia, evolving in the setting of a previous myelodysplastic syndrome.

The patient was treated with induction therapy for acute myelogenous leukemia, from which he achieved a complete response. He then underwent a T-cell depletion allogeneic BMT from a matched sibling donor. At 1.5 months after BMT, a dry, scaly rash without erythema developed bilaterally on his arms, consistent with mild, cutaneous, acute GVHD. He started prednisone therapy at a dosage of 20 mg/d. However, during the next few months, the patient had flaring and generalization of cutaneous GVHD and symptoms of gastrointestinal tract involve-

Transplant	Other Medications	Outcome
Matched URD, T-cell depletion	Prednisone	PUVA response
Matched URD, T-cell depletion	Prednisone, cyclosporine	PUVA response; died
Matched sib, T-cell depletion	Prednisone, cyclosporine	PUVA response
Matched sib	Prednisone, cyclosporine, mycophenolate mofetil	Refractory to ECP
Matched sib, T-cell depletion	Prednisone, cyclosporine	JC virus, died
Matched sib, T-cell depletion	Prednisone, cyclosporine	ECP response; died
Matched sib, T-cell depletion	Prednisone	PUVA response
Matched sib	Prednisone, mycophenolate mofetil	Improved receiving daclizumab
Matched URD	Cyclosporine	Improved receiving cyclosporine
Matched sib	Prednisone, cyclosporine	ECP response; relapse, died
Matched URD	Prednisone, cyclosporine, mycophenolate mofetil	ECP response
Matched sib	Dexamethasone sodium phosphate,† cyclosporine	Improved receiving dexamethasone and cyclosporine
Matched URD	Prednisone, cyclosporine	Improved receiving prednisone and cyclosporine
Matched sib, T-cell depletion	Prednisone, cyclosporine	Relapse, died
Matched URD, T-cell depletion	Prednisone, cyclosporine	Improved receiving prednisone and cyclosporine
Matched sib	Prednisone, mycophenolate mofetil	On waiting list for ECP
Matched URD, T-cell depletion	Prednisone, cyclosporine	Refractory to PUVA, started ECP
Matched sib	Prednisone, cyclosporine, mycophenolate mofetil	ECP response



A, Scaling and erythroderma of chronic cutaneous graft-vs-host disease on the face of a 59-year-old man (patient 5). B, Resolution of these cutaneous symptoms after 3 weeks of treatment with 0.1% tacrolimus ointment twice daily.

ment of the disease when tapering the prednisone dosage. His nausea and diarrhea responded to an increased prednisone dosage of 60 mg/d and initiation of cyclosporine therapy at a dosage of 100 mg twice daily (BID). However, cutaneous GVHD continued to be refractory to this treatment regimen.

The patient was referred to the cutaneous oncology clinic at Dana Farber Cancer Institute 7 months after BMT for further management of chronic cutaneous GVHD. On results of physical examination, he had an impressive degree of generalized erythroderma, with fine scale across the entire head, trunk, and extremities (**Figure** A). There was marked hyperkeratosis on the weight-bearing surfaces of his feet and minimal excoriations on his body. A regimen of a nightly colloidal oatmeal bath, followed by 12% ammonium lactate cream BID and mometasone furoate ointment, had not been helpful. Therefore, at his next follow-up visit, the risks and benefits of topical tacrolimus therapy were discussed with the patient.

The patient was instructed to apply 0.1% tacrolimus ointment twice daily to all affected areas with cutaneous GVHD. After 3 weeks of using tacrolimus ointment, the patient returned to the clinic and reported that his itch and redness were much improved, even in the context of tapering the prednisone dosage recently from 40 to 25 mg/d. Although he had started a BID regimen, he quickly converted to an every-night regimen for convenience. On physical examination, there was marked improvement in the amount of erythema and scaling diffusely, most notably on his face (Figure B), and he had no excoriations. However, the erythema was slightly worse on the upper arms, and there was still a small amount of blanching discoloration on his thighs and knees. The patient was encouraged to continue applying the tacrolimus ointment BID to the affected areas. Three months after initiation of the tacrolimus therapy, the efficacy of this treatment diminished, and in a side-by-side comparison conducted at this time, the benefit of tacrolimus therapy was no longer greater than that of the control vehicle. His cutaneous GVHD appeared

to be stable with a diffuse, mild erythema and scaling in addition to tenderness on the soles of his feet. Because of this loss of efficacy, tacrolimus ointment therapy was discontinued. During the next 3 months, the patient's health deteriorated with a wasting syndrome and worsening of cutaneous GVHD. He was treated with anti–interleukin 2 receptor antibody daclizumab (Zenapax) and became severely immunocompromised. He eventually experienced mental status changes, at which point he was found to have a JC virus infection. The patient died of sepsis 3 months after discontinuation of topical tacrolimus therapy.

COMMENT

The results of this case series suggest that tacrolimus ointment, when applied 2 to 3 times daily, is effective in reducing erythema and pruritus in most patients with systemic steroid-refractory chronic cutaneous GVHD. This case series describes patient reports, physician observations, side-by-side comparisons, and temporal flares to evaluate the benefit of tacrolimus therapy. Because of the observational nature of this case series, no histological evaluation was performed before or after tacrolimus treatment. However, such evaluation should be considered in future, larger-scale studies.

The 2 subtherapeutic serum tacrolimus levels from patients who were responsive to the ointment confirmed that their response was local and that they were not prone to the systemic adverse effects of tacrolimus. Because of its limited profile of adverse effects, tacrolimus ointment could be a useful steroid-sparing therapy for areas of thinner skin, such as the face and genitals. Consistent with previous studies of tacrolimus ointment for other inflammatory skin diseases, this case series showed that patients experienced an effect of tacrolimus, defined as palliation of erythema and pruritus, within hours to days. Although most patients used the topical therapy for about 1 month, 3 patients used it for more than 1 year and found that they continued to have a positive therapeutic response to the drug. Two patients experienced a loss of response to the drug within 3 to 4 months of treatment. It is unclear whether this was due to drug tachyphylaxis or to disease progression beyond the therapeutic efficacy of topical tacrolimus therapy.

As demonstrated by our case report, the natural progression of chronic GVHD can be difficult to control, and despite temporal palliation of cutaneous symptoms with topical tacrolimus, the underlying systemic disease needs to be treated aggressively with an increase in steroid dosage, PUVA therapy, or ECP. Although it appears unlikely to be suitable as a monotherapy for chronic GVHD, tacrolimus ointment may be a safe alternative to topical corticosteroids as a therapeutic bridge to therapies such as PUVA therapy or ECP, which may take months to show efficacy.

Because of the natural waxing and waning nature of the cutaneous symptoms of chronic GVHD, further studies need to be implemented to more definitively correlate topical tacrolimus therapy with alleviation of these symptoms. Future studies will also need to clarify the efficacy of a longer duration of treatment with tacrolimus, its use as an adjunctive treatment to systemic immunosuppressive drugs, and its efficacy in acute GVHD. Given the encouraging results of this preliminary case series, future blinded, randomized, placebo-controlled studies are warranted to investigate the effectiveness of therapy with tacrolimus ointment for cutaneous GVHD.

Accepted for publication May 2, 2001.

This work was supported in part by the American Academy of Dermatology (Schaumburg, Ill) Young Investigator Award (Dr Nghiem).

We would like to thank Stephanie Lee, MD, for her helpful review of the manuscript.

Corresponding author: Paul Nghiem, MD, PhD, Harvard University, Department of Chemistry and Chemical Biology, 12 Oxford St, Cambridge, MA 02138 (e-mail: nghiem@slsiris.harvard.edu).

REFERENCES

1. Atkinson K. Chronic graft-versus-host disease. *Bone Marrow Transplant.* 1990; 5:69-82.
2. Wingard JR, Piantadosi S, Vogelsang GB, et al. Predictors of death from chronic graft-versus-host disease after bone marrow transplantation. *Blood.* 1989;74: 1428-1435.
3. Billingham RE. The biology of graft-versus-host reaction. In: *The Harvey Lectures.* Orlando, Fla: Academic Press Inc; 1966;62:21-78.
4. Ferrara JLM, Deeg HG. Graft-versus-host disease. *N Engl J Med.* 1991;324:667-674.
5. Ferrara JLM. The cytokine modulation of acute graft-versus-host-disease. *Bone Marrow Transplant.* 1998;21(suppl 3):13-15.
6. Antin JH, Ferrara JLM. Cytokine dysregulation and acute graft-versus-host disease. *Blood.* 1992;80:2964-2968.
7. Sullivan KM, Witherspoon RP, Storb R, et al. Alternating-day cyclosporine and prednisone for treatment of high-risk chronic graft-versus-host disease. *Blood.* 1988;72:555-561.
8. Powell FC, Spiegel GT, Muller SA. Treatment of parapsoriasis and mycosis fungoides. *Mayo Clin Proc.* 1984;59:538-546.
9. Edelson R, Berger C, Gasparro F, et al. Treatment of cutaneous T-cell lymphoma by extracoporeal photochemotherapy. *N Engl J Med.* 1987;316:297-303.
10. Wiesmann A, Weller A, Lischka G, Klingebiel T, Kanz L, Einsele H. Treatment of acute graft-versus-host disease with PUVA (psoralen and ultraviolet irradiation): results of a pilot study. *Bone Marrow Transplant.* 1999;23:151-155.
11. Owsianowski M, Gollnick H, Siegert W, et al. Successful treatment of chronic graft-versus-host-disease with extracorporeal photopheresis. *Bone Marrow Transplant.* 1994;14:845-848.
12. Rossetti F, Zulian F, Dall'Amico R, et al. Extracorporeal photo-chemotherapy as single therapy for extensive, cutaneous, chronic graft-versus-host disease. *Transplantation.* 1995;59:149-151.
13. Greineix HT, Volc-Platzer B, Knobler RM. Extracorporeal photochemotherapy in the treatment of severe graft-versus-host-disease. *Leuk Lymphoma.* 2000;36: 425-434.
14. Sawada S, Suzuki G, Kawase Y, Takaku F. Novel immunosuppressive agent, FK506: in vitro effects on cloned T-cell activation. *J Immunol.* 1987;139:1797-1803.
15. Kina T, Hatanaka H, Hashimoto M, et al. FK506, a novel immunosuppressant isolated from a *Streptomyces*, I. *J Antibiot (Tokyo).* 1987;40:1249-1255.
16. Kina T, Hatanaka H, Miyata S, et al. FK506, a novel immunosuppressant isolated from a *Streptomyces*, II. *J Antibiot (Tokyo).* 1987;40:1256-1265.
17. Schreiber SL, Crabtree GR. The mechanism of action of cyclosporine A and FK506. *Immunol Today.* 1992;13:136-142.
18. de Paulis A, Stellato C, Cirillo R, Ciccarelli A, Oriente A, Marone G. Anti-inflammatory effect of FK-506 on human skin mast cells. *J Invest Dermatol.* 1992; 99:723-728.
19. Eberlein-Konig B, Ruzicka T, Michel G, Przybilla B. Modulation of histamine release in vitro by FK506 and interleukin-3 is determined by sequence of incubation. *Arch Dermatol Res.* 1997;289:606-608.
20. Ruzicka T, Bieber T, Schopf E, et al. A short-term trial of tacrolimus ointment for atopic dermatitis. *N Engl J Med.* 1997;337:816-821.
21. Reitamo S, Wollenberg A, Schopf E, et al. Safety and efficacy of 1 year of tacrolimus ointment monotherapy in adults with atopic dermatitis. *Arch Dermatol.* 2000; 136:999-1006.
22. The European FK506 Multicentre Psoriasis Study Group. Systemic tacrolimus (FK506) is effective for the treatment of psoriasis in a double-blind, placebo-controlled study. *Arch Dermatol.* 1996;132:419-423.
23. Reitamo S, Rissanen J, Remitz A, et al. Tacrolimus ointment does not affect collagen synthesis. *J Invest Dermatol.* 1998;111:396-398.
24. Kawashima M, Nakagawa H, Ohtsuki M, Tamaki K, Ishibashi Y. Tacrolimus concentrations in blood during topical treatment of atopic dermatitis. *Lancet.* 1996; 348:1240-1241.
25. Aoyama H, Tabata N, Tanaka M, Uesugi Y, Tagami H. Successful treatment of resistant facial lesions of atopic dermatitis with 0.1% FK506 ointment. *Br J Dermatol.* 1995;133:494-496.