The ascomycin macrolactam pimecrolimus (Elidel, SDZ ASM 981) is a potent inhibitor of mediator release from human dermal mast cells and peripheral blood basophils

Torsten Zuberbier, MD,[a] Sie-Uen Chong,[a] Karl Grunow, MD,[a] Sven Guhl,[a] Pia Welker, PhD,[a] Maximilian Grassberger, PhD,[b] and Beate M. Henz, MD[a]

Berlin, Germany, and Vienna, Austria

Background: The ascomycin macrolactam pimecrolimus (Elidel, SDZ ASM 981) has recently been developed as a novel and cell-selective inhibitor of inflammatory cytokine secretion; it has fewer adverse effects than currently available drugs.
Objective: In this study, we investigated the capacity of pimecrolimus to directly inhibit in vitro mediator release from human skin mast cells and basophils.
Methods: Purified cutaneous mast cells or basophil-containing peripheral blood leukocytes were obtained from healthy human donors and preincubated with pimecrolimus (0.1 nmol/L to 1 μmol/L) in the absence or presence of its specific antagonist (rapamycin), cyclosporin A (100 nmol/L to 1 μmol/L), or dexamethasone (1 μmol/L) and then stimulated with anti-IgE or with calcium ionophore A23187 plus phorbol myristate acetate. Cell supernatants were kept for analysis of histamine, tryptase, LTC4, and TNF-α.
Results: Pimecrolimus caused a strong and dose-dependent inhibition of anti-IgE–induced release of histamine from mast cells and basophils (maximally 73% and 82%, respectively, at 500 nmol/L pimecrolimus) and of mast cell tryptase (maximally 75%) and a less pronounced inhibition of LTC4 (maximally 32%) and of calcium ionophore plus phorbol myristate acetate–induced mast cell TNF-α release (90% maximum at 100 nmol/L pimecrolimus). In contrast, inhibition achieved during mast cell histamine release was maximally 60% with cyclosporin A and only 28% with dexamethasone.
Conclusion: These data demonstrate a marked inhibitory capacity of pimecrolimus on mediator release from human mast cells and basophils with a potency exceeding that of cyclosporin A and dexamethasone. Pimecrolimus might thus be expected to be effective in the treatment of mast cell– and basophil-dependent diseases. (J Allergy Clin Immunol 2001;108:275-80.)

Key words: *Mast cell, allergy, histamine, SDZ ASM 981, pimecrolimus, macrophilin, ascomycin*

Abbreviations used

SDZ ASM 981:	Prototype name for a newly developed macrolactam
PMA:	Phorbol myristate acetate
LTC4:	Leukotriene C4
DMEM:	Dulbecco modified Eagle medium
MACS:	Magnetic cell sorting

Type I allergic diseases are frequent in the general population and show an increasing prevalence. Although mast cells and basophils are the main effector cells in these diseases, most therapeutic approaches in allergy currently concentrate on antagonizing the effects of mast cell–derived mediators, especially histamine. Although cyclosporin A[1] and tacrolimus (FK506)[2] have been described as exerting a direct inhibitory effect on mast cell mediator release at higher concentrations, they are limited in their usefulness because of their pharmacologic profile. Thus, novel therapeutic alternatives need to be investigated for their potential efficacy and with regard to their direct inhibitory effects on mast cell and basophil activation.

The ascomycin macrolactam pimecrolimus is a novel anti-inflammatory drug that was developed specifically for the treatment of inflammatory skin diseases.[3] It binds to macrophilin and inhibits the phosphatase calcineurin. As a consequence, it selectively suppresses the production of inflammatory cytokines in T cells and T-cell proliferation.[4] Pimecrolimus was also shown to inhibit the release of proinflammatory mediators, including TNF-α, in the rodent basophil cell line RBL 2H3 after anti-IgE–induced activation. This effect was antagonized by rapamycin, which competitively binds to macrophilin but does not itself affect the anti-IgE–stimulated activation of mast cells.[5] In vivo, pimecrolimus exhibits marked activity in animal models of inflammatory skin diseases such as allergic contact dermatitis[6] and magnesium-deficient diet-induced dermatitis.[7] Unlike corticosteroids, topical pimecrolimus has no atrophogenic potential.[4] In contrast to cyclosporin A and tacrolimus, pimecrolimus has a low potential for affecting systemic immune responses.[4,8] In the clinic, topical pimecrolimus proved to be effective in

From [a]the Department of Dermatology and Allergy, Charité, Humboldt University Berlin; and [b]Novartis Forschungs Institut, Vienna.
Received for publication November 20, 2000; revised April 19, 2001; accepted for publication April 26, 2001.
Reprint requests: Torsten Zuberbier, MD, Department of Dermatology and Allergy, Charité, Schumannstr. 20/21, D-10117 Berlin, Germany.

0091-6749/2001 $35.00 + 0 **1/85/116865**
doi:10.1067/mai.2001.116865

patients with atopic dermatitis (children and adults), allergic contact dermatitis, irritant contact dermatitis, and—under occlusion—plaque psoriasis.[9] In a pilot study, oral pimecrolimus proved to be highly efficient in the treatment of moderate to severe psoriasis.[10]

To further explore the mechanism of pimecrolimus action in skin diseases, we have investigated its effect on in vitro mediator release from human dermal mast cells as well as basophils.

MATERIALS AND METHODS

Reagents

The following reagents were purchased: BSA, PIPES, monothioglycerol, hyaluronidase type I-S, dexamethasone (Sigma, Deisenhofen, Germany), collagenase type I, serum goat anti–human IgE (Behring, Marburg, Germany), DNase type I (Boehringer, Mannheim, Germany), calcium ionophore A23187 (Calbiochem, Bad Soden, Germany), leukotriene C4 (LTC4) EIA (Cayman, Ann Arbor, USA), and TNF-α ELISA (R&D Systems, Wiesbaden, Germany). Cyclosporin A and pimecrolimus were from Novartis (Vienna, Austria).

FCS, horse serum, RPMI-1640, Basal Iscove's medium, amphotericin B, L-glutamine (Biochrom Seromed, Berlin, Germany), DMEM (Gibco BRL, Karlsruhe, Germany), D-glucose, NaCl, KCl (Merck, Darmstadt, Germany), dextran T500 (Pharmacia, Upsala, Sweden), PBS w/o (PAA, Linz, Austria), goat antimouse microbeads, separation columns (Miltenyi Biotech, Bergisch Gladbach, Germany), and monoclonal antibody Yb5B8 (CD117; a kind gift of L.K. Ashman, Adelaide, Australia) were also used.

Buffers and media

The PAG-CM buffer consisted of PIPES 250 mmol/L, 1.19 mol/L NaCl, 50 mmol/L KCl, 0.025% human serum albumin, 1% glucose, 3 mmol/L Ca^{2+}, and 3 mmol/L Mg^{2+}, adjusted to pH 7.4.

The digestion buffer consisted of RPMI 1640 supplemented with 2% FCS, 1% penicillin/streptomycin, amphotericin B 2.5 mg/L, 1% L-glutamine, 5 nmol/L $MgSO_4$, DNase type I 10 μg/mL, and 10 μL monothioglycerol.

The mast cell medium consisted of DMEM, 10% FCS, 10% horse serum, 1% penicillin/streptomycin, amphotericin B 2.5 mg/L, 1% L-glutamine, NEAA (5 mL/500 mL), and 10 μL monothioglycerol.

The TNF-α release medium consisted of Basal Iscove's modified DMEM containing 0.1% BSA.

Isolation of human skin mast cells

Mast cells from normal human breast skin (from plastic surgery after informed consent) were isolated by enzymatic dispersion, as described previously (with minor modifications).[11] Briefly, skin was cut into small pieces (1-2 mm^2), dispersed with collagenase and hyaluronidase (15 mg and 7.5 mg/g of tissue, respectively, in 5- to 10-mL digestion medium/g tissue) in two 1-hour cycles, separated from undissociated tissue by filtration through nylon gauze (300, 100, and 40 μm), and then washed twice in PBS. Viability and total cell number were examined in cells exposed to trypan blue, and mast cell purity was assessed by metachromasia of cells on exposure to toluidine blue. The procedure yielded 6 to 8×10^5 mast cells/10 g of wet weight tissue, 3% to 5% of all nucleated cells being mast cells and more than 95% cells being viable. Unpurified cells were used in histamine and tryptase release experiments because dermal mast cells are the sole source of these mediators. For experiments pertaining to LTC4 and TNF-α release, mast cells were further purified by the magnetic cell sorting (MACS) technique (purity, 85% to 95%). Briefly, after the mast cells were labeled with monoclonal c-kit antibody[12] and a secondary goat antimouse was attached for magnetic microbeads, mast cells were retained in the magnetic field of the MACS column and elutriated with PBS.

Preparation of leukocytes for basophil histamine release assay

Twenty milliliters of venous blood were obtained from each of several healthy volunteers (who had given informed consent) and placed into polypropylene tubes containing 2 mL dextran T500 6% in 0.9% NaCl and 2 mL 100 mmol/L EDTA. After sedimentation (30-45 minutes at 22°C), the leukocyte-containing upper layer was removed and centrifuged (4°C for 8 minutes; 130*g*). The cells were then washed twice (in PBS) and resuspended in PAG-CM.

Preincubation with pimecrolimus

Mast cells or leukocytes were preincubated for 60 minutes at 37°C with pimecrolimus dissolved in PAG-CM containing 0.02% dimethyl sulfoxide. The cells were then washed twice in PBS and stimulated, as described below. Controls were preincubated in the same dimethyl sulfoxide containing buffer only. In pilot experiments, more prolonged times of preincubation with pimecrolimus (1, 2, and 4 h) had been investigated; no differences were demonstrated.

Mediator release

Aliquots of mast cells (2×10^4/mL) or basophils (7×10^5/mL, admixed with lymphocytes) in PAG-CM were challenged with a previously established optimal dose of anti-IgE (2000 IU/mL for mast cells and 200 IU/mL for basophils). Spontaneous release was measured by the addition of PAG-CM instead of stimuli, and the total amount of cellular histamine was measured after lysis of cells with 2% perchloric acid. After a 30-minute incubation at 37°C, samples were centrifuged at 300*g*; supernatants were kept at –20°C until measurement. All experiments were performed in duplicate. Histamine release was expressed as percent release of total histamine after subtraction of spontaneous release.

Tryptase activity of mast cells was expressed as enzyme released into the supernatant over total intracellular amount, as determined in samples exposed to 3 cycles of freezing and thawing.

LTC4 was measured in supernatants of mast cells (2×10^4/mL) challenged for 10 minutes at 37°C with anti-IgE (2000 U/mL). After centrifugation, samples were stored at –70°C until analysis.

For TNF-α experiments, purified mast cells (1×10^6/mL) were precultured for 24 hours in Basal Iscove's medium containing 1% BSA and then stimulated for 24 hours in the same medium with 0.1% BSA, 0.2 μmol/L A23187 and 20 nmol/L phorbol myristate acetate (PMA) being added for stimulation of cells.

Cell free supernatants were shock-frozen and stored at –70°C.

Mediator assays

Histamine was measured through use of the automatic fluorometric method described by Siraganian.[13] TNF-α and LTC4 were determined by means of a commercial ELISA/EIA kit. Tryptase activity was assessed by measuring specific substrate cleavage spectrometrically, as described previously.[14]

Statistics

The nonparametric Wilcoxon test for paired data was used throughout.[15]

RESULTS

Preincubation of human dermal mast cells with pimecrolimus showed a dose-dependent, statistically significant inhibition of anti-IgE–induced histamine release

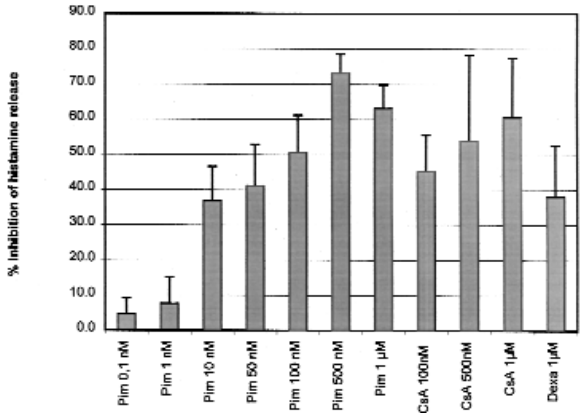

FIG 1. Anti-IgE–induced (2000 IU/mL) histamine release from human dermal mast cells preincubated with pimecrolimus (*Pim*; mean ± SEM, n = 10, histamine release of controls = 13% ± 1.6% of total histamine, $P < .01$ for concentrations of ≥10 nmol/L pimecrolimus), cyclosporin A (*CsA*), and dexamethasone (*Dexa*; mean ± SEM; n = 3; histamine release of controls = 7.6% ± 1.5% of total histamine).

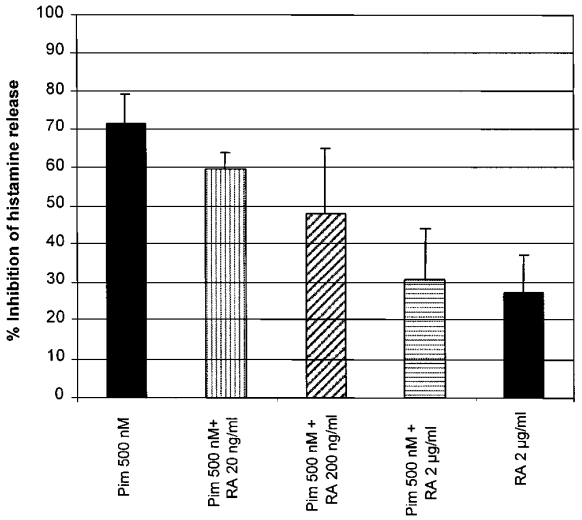

FIG 2. Effect of the pimecrolimus (*Pim*) antagonist rapamycin (*RA*) on pimecrolimus during anti-IgE–induced (2000 IU/mL) histamine release (mean ± SEM; n = 5; histamine release of controls = 10.4% ± 1.9 of total histamine; $P > .05$).

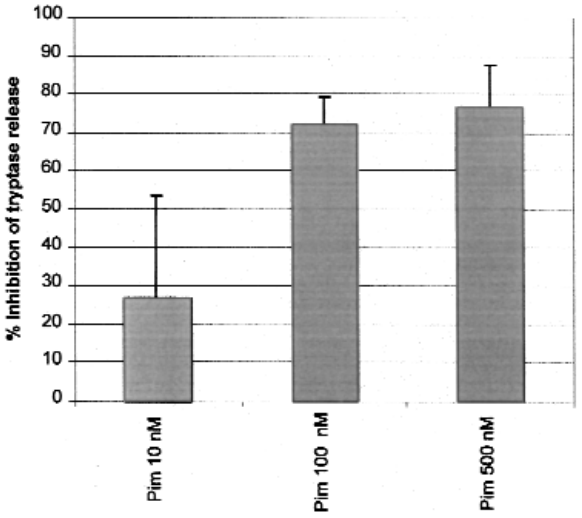

FIG 3. Dose-dependent effect of pimecrolimus (*Pim*) on anti-IgE–induced (2000 IU/mL) tryptase release from dermal mast cells (mean ± SEM; n = 3).

(mean histamine release of controls, 13% ± 1.6% of total histamine after deduction of spontaneous release; Fig 1), whereas there was no influence on spontaneous histamine release (mean, 7.4% ± 1.3%). Maximal inhibition (72%) by pimecrolimus was observed at a concentration of 500 nmol/L, but significant inhibition was still seen at concentrations as low as 10 nmol/L (mean inhibition, 38%). The inhibitory capacity of pimecrolimus exceeded that of cyclosporin A and dexamethasone, which showed maximal histamine release inhibitions of 60% and 37%, respectively, at 1 µmol/L (Fig 1).

To investigate the specificity of the observed inhibitory effect, pimecrolimus and its specific antagonist, rapamycin, were added simultaneously to the cells. Although rapamycin exerted a moderately inhibitory effect by itself, it was also able to dose-dependently block the inhibitory activity of pimecrolimus (Fig 2).

In the same mast cell supernatants, the influence of pimecrolimus on anti-IgE–mediated release was also investigated on mast cell tryptase release and on de novo generation of LTC4. Tryptase release was inhibited to an extent similar to that seen with histamine (Fig 3), whereas effects on LTC4 release were less pronounced (Fig 4).

In an additional set of experiments, the influence of pimecrolimus on mast cell–derived TNF-α was investigated. Because in preliminary experiments only small amounts of TNF-α, slightly exceeding the detection level of the ELISA, were generated on stimulation of dermal mast cells with anti-IgE, highly purified mast cells were stimulated with calcium ionophore and PMA instead. As with histamine and tryptase, TNF-α release was strongly and dose-dependently inhibited (>80%) by pimecrolimus with this stimulus (Fig 5).

On examination of basophil leukocytes as additional effector cells of anaphylactic reactions, an inhibitory effect of pimecrolimus similar to that seen with histamine from purified dermal mast cells could be demonstrated (Fig 6) at the same maximal concentration of pimecrolimus studied (see Fig 1 for comparison). As in mast cells, the spontaneous histamine release remained unchanged.

DISCUSSION

Currently, most therapeutic approaches in allergy concentrate on antagonizing the effects of mast cell– and basophil-derived mediators. Corticosteroids, cyclosporin A, and tacrolimus, which also efficiently inhibit mast cell mediator release, are limited in their use because of their pharmacologic profile.

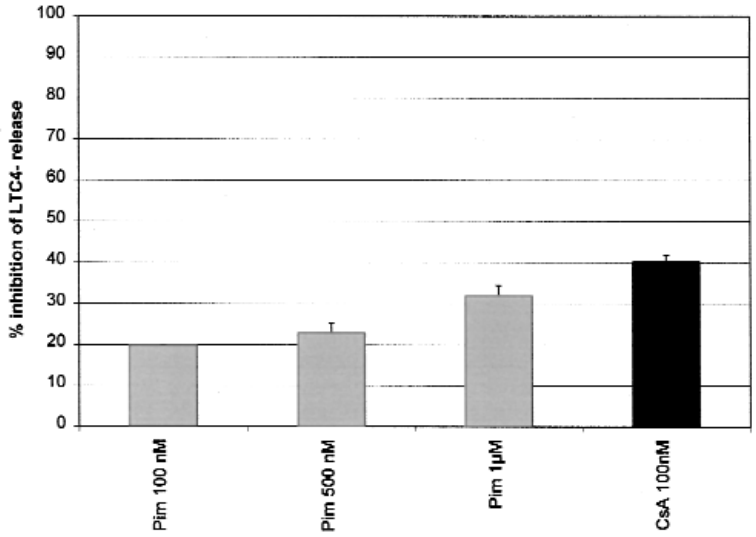

FIG 4. Dose-dependent influence of pimecrolimus (*Pim*) and cyclosporin A (*CsA*; 100 nmol/L) on anti-IgE–induced (2000 IU/mL) LTC4 release from dermal mast cells (mean ± SEM; n = 3; LTC4 release of controls = 63 ± 21 pg/mL).

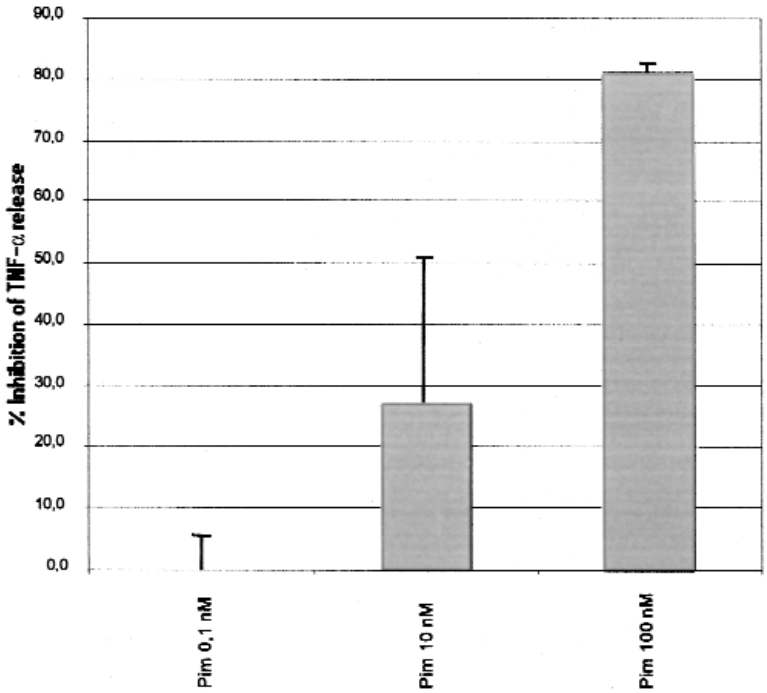

FIG 5. Dose-dependent effect of pimecrolimus (*Pim*) on calcium ionophore A23187 (0.2 μmol/L) plus PMA–induced (20 ng/mL) TNF-α release from purified dermal mast cells (mean ± SEM; n = 4; TNF-α release of controls = 508 ± 134 pg/mL).

In the present study, we have shown for the first time that pimecrolimus has a direct and specific effect on the release of several proinflammatory mediators from human mast cells as well as on histamine release from human basophils; furthermore, inasmuch as in both mast cells and basophils spontaneous histamine release remained unaffected, a toxic effect is unlikely. The effect of rapamycin on the inhibition of the anti-IgE–stimulated histamine release was studied to evaluate whether the effect of pimecrolimus is macrophilin-dependent. Rapamycin shares with ascomycin a structural element ("binding domain") that mediates the binding to macrophilin, the common cytosolic receptor for the immunomodulating macrolides.[16] Rapamycin and

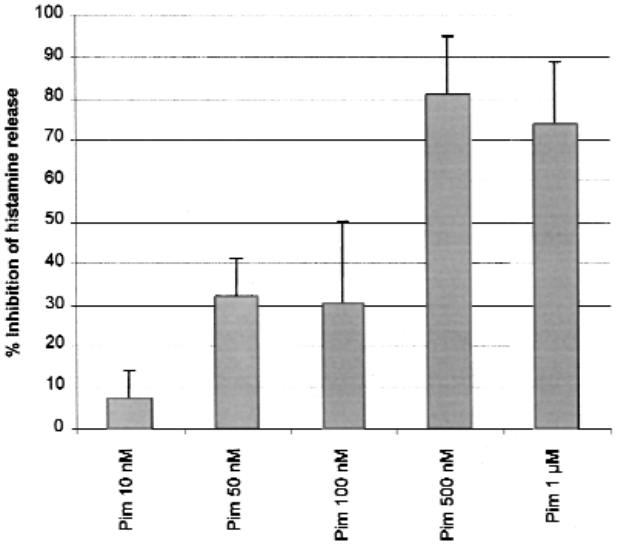

FIG 6. Dose-dependent effect of pimecrolimus (*Pim*) on anti-IgE–induced (200 IU/mL) histamine release from human basophils (mean ± SEM; n = 3; histamine release of controls = 28.3% ± 5.4% of total histamine).

506BD, a semisynthetic FK506 derivative representing the "binding domain" in essence, have been shown to bind to macrophilin and to suppress its rotamase activity but to have no effect on calcineurin, the target of the ascomycin-macrophilin complex.[17,18] Rapamycin and 506BD proved also not to affect the IgE receptor–mediated exocytosis in the rat basophilic leukemia cell line RBL-2H3.[19] However, they suppressed the inhibition of exocytosis by FK506 but not by cyclosporin A—these being 2 immunomodulators that inhibit the phosphatase activity of calcineurin but bind to different immunophilins (FK506 to macrophilin, cyclosporin A to cyclophilin). The antagonizing effect was therefore explained by the competitive binding of 506BD or rapamycin to macrophilin, thus preventing the formation of an active FK506-macrophilin complex. Rapamycin was similarly shown to antagonize the effect of pimecrolimus in RBL-2H3 cells.[5] This has now been confirmed in human dermal mast cells, supporting the concept that the action of pimecrolimus is specific and macrophilin-dependent.

Of the numerous mast cell mediators, histamine is generally regarded as the main inducer of allergic reactions—a point of view clearly sustained by the excellent efficacy of antihistamines in urticaria and allergic rhinitis. Recently, however, evidence has been growing that mast cells promote allergic reactions not only through the release of histamine but also by generating and secreting a plethora of other proinflammatory mediators and cytokines.[20] Among these, the lipid mediator LTC4, the enzyme tryptase, and the cytokine TNF-α were chosen for study as representatives of the different chemical classes of mediators, in part because of their special pathologic and clinical relevance in allergic reactions.

The inhibition of IgE-dependent release of the mast cell–specific protease tryptase by pimecrolimus is of special interest, inasmuch as there is recent growing evidence that tryptase plays a very specific role in allergic inflammation. Tryptase can induce the release of active IL-1β and IL-8 from human endothelial cells.[21] In the guinea pig, intradermally injected human mast cell tryptase resulted in the accumulation of large numbers of neutrophils and eosinophils[22]; the anaphylatoxin C3a was shown to be generated by human mast cell tryptase after cleavage of C3[23]; and it was recently demonstrated that purified mast cell tryptase enhances human bronchial hyperreactivity to histamine.[24]

Like the other mast cell mediators inhibited by pimecrolimus, TNF-α is a potent proinflammatory cytokine that is partly preformed within human mast cells. Although a systemic increase in soluble TNF-α was not observed in atopic patients,[25,26] an increase in soluble TNF-α receptor has been measured during the pollen season in allergic rhinitis,[25] underlining the possible relevance of this cytokine in allergy. Furthermore, immunoreactivity for TNF-α has been observed in mast cells of patients with atopic dermatitis in lesional skin as well as of urticaria patients in nonlesional skin.[27,28] TNF-α is also known to induce the expression of the endothelial adhesion molecules ICAM-1 and ELAM-1, thus enhancing the acute and late-phase leukocyte infiltration into inflamed tissue.[29] Apparently, mast cells are also able to enhance survival of tissue eosinophils via TNF-α, because anti–TNF-α neutralizing antibodies can reduce this effect.[30]

LTC4 differs from the other mediators studied here in that inhibition by pimecrolimus is less pronounced and the inhibitory potency is lower than that seen in cyclosporin A. It is also lower than the inhibition described in the literature for FK506.[31] Because all mast cell mediators are highly divergent in their chemical natures and mechanisms of synthesis, reasons for this lower inhibition might be various and are not readily dis-

cernible. In contrast to preformed histamine, tryptase, and perhaps TNF-α, LTC4 is generated de novo and rapidly released by both basophils and mast cells through 5-lipoxygenase–mediated cleavage of membrane-bound arachidonic acid.[20]

The effect of pimecrolimus on mast cells and basophils, as reported here, is more pronounced than that of dexamethasone or cyclosporin A. The only moderate effect of corticosteroids on the control of type I allergic symptoms is well known in patients with allergic rhinitis, as well as in patients with urticaria. In general, doses above 40 mg of prednisolone are required for the control of histamine-dependent symptoms such as whealing and rhinorrhea. Because adverse effects are inevitable at this dosage in prolonged treatment, systemic corticosteroids are preferably avoided for the treatment of allergic diseases. With the development of pimecrolimus, a novel drug is available that does not seem to be associated with the side effects seen with currently used drugs. As demonstrated in animals, pimecrolimus has a low potential to affect systemic immune responses after oral or parenteral administration.[4,8,32] In a pilot study in patients with psoriasis, oral pimecrolimus proved to be well tolerated and safe up to the highest dose tested (60 mg/day). Pimecrolimus has the potential of being a highly specific antagonist of mast cell mediator release, with a low adverse effect profile, after topical or oral administration. Patients with immediate and late-type allergic diseases, as well as patients with other mast cell–associated conditions (such as mastocytosis), might benefit from this drug.

REFERENCES

1. Stellato C, De Paulis A, Ciccarelli A, Cirillo R, Patella V, Casolaro V, et al. Anti-inflammatory effect of cyclosporin A on human skin mast cells. J Invest Dermatol 1992;98:800-4.
2. 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;99:723-8.
3. Paul C, Graeber M, Stuetz A. Ascomycins: promising agents for the treatment of inflammatory skin diseases. Expert Opin Invest Drugs 2000;9:69-77.
4. Grassberger M, Baumruker T, Enz A, Hiestand P, Hultsch T, Kalthoff F, et al. A novel anti-inflammatory drug, SDZ ASM 981, for the treatment of skin diseases: in vitro pharmacology. Br J Dermatol 1999;141:264-73.
5. Hultsch T, Muller KD, Meingassner JG, Grassberger M, Schopf RE, Knop J. Ascomycin macrolactam derivative SDZ ASM 981 inhibits the release of granule-associated mediators and of newly synthesized cytokines in RBL 2H3 mast cells in an immunophilin-dependent manner. Arch Dermatol Res 1998;290:501-7.
6. Meingassner JG, Grassberger M, Fahrngruber H, Moore HD, Schuurmann H, Stütz A. A novel anti-inflammatory drug, SDZ ASM 981, for the topical and oral treatment of skin diseases: in vivo pharmacology. Br J Dermatol 1997;137:568-76.
7. Neckermann G, Bavandi A, Meingassner JG. Atopic dermatitis-like symptoms in hypomagnesemic hairless rats are prevented and inhibited by sytemic or topical SDZ ASM 981. Br J Dermatol 2000;142:669-79.
8. Stuetz A. SDZ ASM 981: Mode of action and pharmacological selectivity. J Eur Acad Dermatol Venereol. In press.
9. Paul C, Ho VC. Ascomycins in dermatology. Semin Cutan Med Surg 1998;4:256-9.
10. Rappersberger K, Komar M, Ebelin ME, Scott G, Bueche M, Burtin P, et al. Oral SDZ ASM 981: safety, pharmacokinetics and efficacy in patients with moderate to severe chronic plaque psoriasis [abstract]. J Invest Dermatol 2000;114:776.
11. Lowman MA, Rees PH, Benyon RC, Church MK. Human mast cell heterogeneity: histamine release from mast cells dispersed from skin, lung, adenoids, tonsils, and colon in response to IgE-dependent and nonimmunologic stimuli. J Allergy Clin Immunol 1988;81:590-7.
12. Lerner NB, Nocka KH, Cole SR, Qiu FH, Strife A, Ashman LK, et al. Monoclonal antibody YB5.B8 identifies the human c-kit protein product. Blood 1991;77:1876-83.
13. Siraganian RP. Refinements in the automated fluorometric histamine analysis system. J Immunol Methods 1975;7:283-90.
14. Harvima IT, Schechter NM, Harvima RJ, Fraki JE. Human skin tryptase: purification, partial characterization and comparison with human lung tryptase. Biochim Biophys Acta 1988;957:71-80.
15. Noether GE. Introduction to statistics. The nonparametric way. New York: Springer; 1991.
16. Schreiber SL. Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science 1991;251:283-7.
17. Bierer BE, Somers PK, Wandless TJ, Burakoff SJ, Schreiber SL. Probing immunosuppressant action with a nonnatural immunophilin ligand. Science 1990;250:556-9.
18. Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 1991;66:807-15.
19. Hultsch T, Albers MW, Schreiber SL, Hohman RJ. Immunophilin ligands demonstrate common features of signal transduction leading to exocytosis or transcription. Proc Natl Acad Sci U S A 1991;88:6229-33.
20. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev 1997;77:1033-79.
21. Compton SJ, Cairns JA, Holgate ST, Walls AF. The role of mast cell tryptase in regulating endothelial cell proliferation, cytokine release, and adhesion molecule expression: tryptase induces expression of mRNA for IL-1 beta and IL-8 and stimulates the selective release of IL-8 from human umbilical vein endothelial cells. J Immunol 1998;161:1939-46.
22. He S, Peng Q, Walls AF. Potent induction of a neutrophil and eosinophil-rich infiltrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. J Immunol 1997;159:6216-25.
23. Schwartz LB, Kawahara MS, Hugli TE, Vik D, Fearon DT, Austen KF. Generation of C3a anaphylatoxin from human C3 by human mast cell tryptase. J Immunol 1983;130:1891-5.
24. Berger P, Compton SJ, Molimard M, Walls AF, N'Guyen C, Marthan R, et al. Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. Clin Exp Allergy 1999;29:804-12.
25. Kato M, Hattori T, Kato Y, Matsumoto Y, Yamashita T, Nakashima I. Elevated soluble tumor necrosis factor receptor levels in seasonal allergic rhinitis patients. Allergy 1999;54:278-82.
26. Laan MP, Koning H, Baert MR, Oranje AP, Buurman WA, Savelkoul HF, et al. Levels of soluble intercellular adhesion molecule-1, soluble E-selectin, tumor necrosis factor-alpha, and soluble tumor necrosis factor receptor p55 and p75 in atopic children. Allergy 1998;53:51-8.
27. Ackermann L, Harvima IT. Mast cells of psoriatic and atopic dermatitis skin are positive for TNF-alpha and their degranulation is associated with expression of ICAM-1 in the epidermis. Arch Dermatol Res 1998;290:353-9.
28. Hermes B, Prochazka A-K, Haas N, Jurgovsky K, Sticherling M, Henz BM. Upregulation of tumor necrosis factor α and interleukin-3 expression in lesional and uninvolved skin in different types of urticaria. J Allergy Clin Immunol 1999;103:307-14.
29. Pearlman DS. Pathophysiology of the inflammatory response. J Allergy Clin Immunol 1999;104:S132-S137.
30. Levi-Schaffer F, Temkin V, Malamud V, Feld S, Zilberman Y. Mast cells enhance eosinophil survival in vitro: role of TNF-alpha and granulocyte-macrophage colony-stimulating factor. J Immunol 1998;160:5554-62.
31. De Paulis A, Ciccarelli A, Marino I, De Crescenzo G, Marino D, Marone G. Human synovial mast cells. II. Heterogeneity of the pharmalogical effects of antiinflammatory and immunosuppressive drugs. Arthritis Rheum 1997;40:469-78.
32. Meingassner J, Fahrngruber H, Bavandi A. SDZ ASM 981, in contrast to CyA and FK 506, does not suppress the primary immune response in murine allergic contact dermatitis [conference abstract]. J Invest Dermatol 2000;114:832.