

# Effects of cyclosporin A and FK-506 on stem cell factor-induced histamine secretion and growth of human mast cells

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*Stem cell factor (SCF) is a key regulator of human mast cells (MCs) and a potential mediator of allergy. In this study the effects of cyclosporin A (CSA) and FK-506, two potent immunosuppressive drugs, on SCF-dependent histamine release and growth of human MCs were analyzed. Preincubation of tissue MCs with CSA (3 µg/ml) resulted in inhibition of histamine release provoked by either recombinant human (rh) SCF (70.3% ± 20.6% inhibition,  $p < 0.001$ ) or anti-IgE (76.7% ± 21.9%,  $p < 0.001$ ) or by rhSCF + anti-IgE (77.4% ± 13.9%,  $p < 0.001$ ). Almost the same inhibition was produced by FK-506 (rhSCF: 82.0% ± 18.9% inhibition,  $p < 0.001$ ; anti-IgE: 71.5% ± 16.7%,  $p < 0.001$ ; rhSCF + anti-IgE: 70.0% ± 7.3%,  $p < 0.001$ ). The effects of CSA and FK-506 on SCF-dependent release of histamine were dose-dependent ( $IC_{50}$ : CSA, 1 to 10 ng/ml; FK-506, 0.3 to 3 ng/ml).  $IC_{50}$  values about three to 10 times higher were found for MCs preincubated with rhSCF before anti-IgE activation, compared with anti-IgE or SCF alone. SCF-dependent differentiation of human MCs was analyzed in a long-term suspension culture system ( $n = 6$ ). Unexpectedly, CSA and FK-506 were unable to suppress, but even enhanced SCF-dependent growth of MCs and formation of MC tryptase in long-term culture. Together, CSA and FK-506 inhibit SCF-dependent release of histamine from human MCs and even augment SCF-dependent growth of human MCs in long-term culture. (J Allergy Clin Immunol 1996;98:389-99.)*

**Key words:** Mast cells, histamine, c-kit, stem cell factor, IgE, tryptase

Mast cells (MCs) are effector cells of allergic and other inflammatory reactions.<sup>1-3</sup> They originate from hemopoietic progenitor cells,<sup>4</sup> but in contrast to other hemopoietic cells, are located in extravascular areas. Differentiation and growth of human MCs from their progenitor cells can be

## Abbreviations used

cAMP:	Cyclic adenosine monophosphate
CSA:	Cyclosporin A
CSH:	Cyclosporin H
FCS:	Fetal calf serum
$IC_{50}$ :	Inhibitory concentration of 50%
LTC:	Long-term culture
mAb:	Monoclonal antibody
MC:	Mast cell
SCF:	Stem cell factor

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induced in long-term culture (LTC) by the stroma cell product, stem cell factor (SCF).<sup>5,6</sup> SCF, also termed *kit ligand*, *steel factor*, or *mast cell growth factor*, is expressed in either soluble or membrane-bound form.<sup>7-9</sup> However, SCF is not only a growth factor but also a specific and potent activation factor for MCs and thus a potential mediator of allergy. In particular, recombinant SCF promotes

the capacity of human MCs to release vasoactive mediators and co-operates with IgE-dependent cell activation in MCs.<sup>5, 10-12</sup> In addition, SCF per se is capable of inducing release of mediators from human or rodent MCs.<sup>11-13</sup> The effects of SCF on MCs are mediated through a transmembrane tyrosine kinase receptor, the product of the c-kit proto-oncogene.<sup>10-17</sup> Receptors for SCF are expressed on immature hemopoietic precursor cells,<sup>18</sup> on the MC progenitor cell line HMC-1,<sup>16</sup> and on mature MCs.<sup>5, 12, 14</sup>

Cyclosporin A (CSA) and FK-506 are two potent immunosuppressive drugs used in transplantation medicine and treatment of inflammatory disease states.<sup>19-27</sup> Both drugs have been shown to exert antiinflammatory effects on a variety of immune cells including T cells, basophils, and MCs.<sup>23, 24, 26, 28-37</sup> A number of studies have shown that CSA and FK-506 inhibit cytokine-dependent growth and function of T cells<sup>19, 23-26, 35-37</sup> and IgE-dependent mediator release from basophils and MCs.<sup>28-34</sup> Interestingly, in human basophils, IL-3 reversed the inhibitory effect of CSA and FK-506.<sup>29</sup> However, the effects of CSA and FK-506 on cytokine-dependent functions of human MCs have so far not been presented. The aim of this study was to analyze the effects of CSA and FK-506 on SCF-dependent growth and function of human MCs.

## METHODS

### Reagents

Collagenase (type II) was purchased from Worthington Biochemical Corporation (Freehold, N.J.). One liter of  $Mg^{++}$  and  $Ca^{++}$  free Tyrode's buffer contained 0.2 gm of KCl, 0.05 gm of  $NaH_2PO_4 \cdot H_2O$ , 8.0 gm of NaCl, and 1 gm of glucose (anhydrous). PIPES buffer contained 25 mmol/L piperazine-N,N'-bis[2-ethanesulfonic acid], 110 mmol/L NaCl, 5 mmol/L KCl, and 2.0 mmol/L  $CaCl_2$  (pH 7.35). CSA, cyclosporin H (CSH), FK-506, recombinant human (rh)IL-4, and rhIL-3 were provided by Sandoz Vienna (Vienna, Austria). Recombinant human SCF was provided by Amgen (Thousand Oaks, Calif.). Phorbol 12-myristate 13-acetate and calcium ionophore A-23187 were from Sigma Chemical Company (St. Louis, Mo.). The monoclonal antibodies (mAbs) BA-2 (CD9), E-124-2-8 [D $\epsilon$ 2] (anti-IgE), and CLB-gran/12 (CD63) were purchased from Immunotech (Marseille, France). The mAbs G19-1 (CD43) and F10-44-2 (CD44) were obtained from the 4th International Workshop on Human Leukocyte Differentiation Antigens, Vienna 1989. Monoclonal IgE was purchased from Chemicon (Temecula, Calif.). The anti-c-kit mAb YB5.B8<sup>14, 15</sup> was a kind gift from L. K. Ashman (University of Adelaide, Australia).

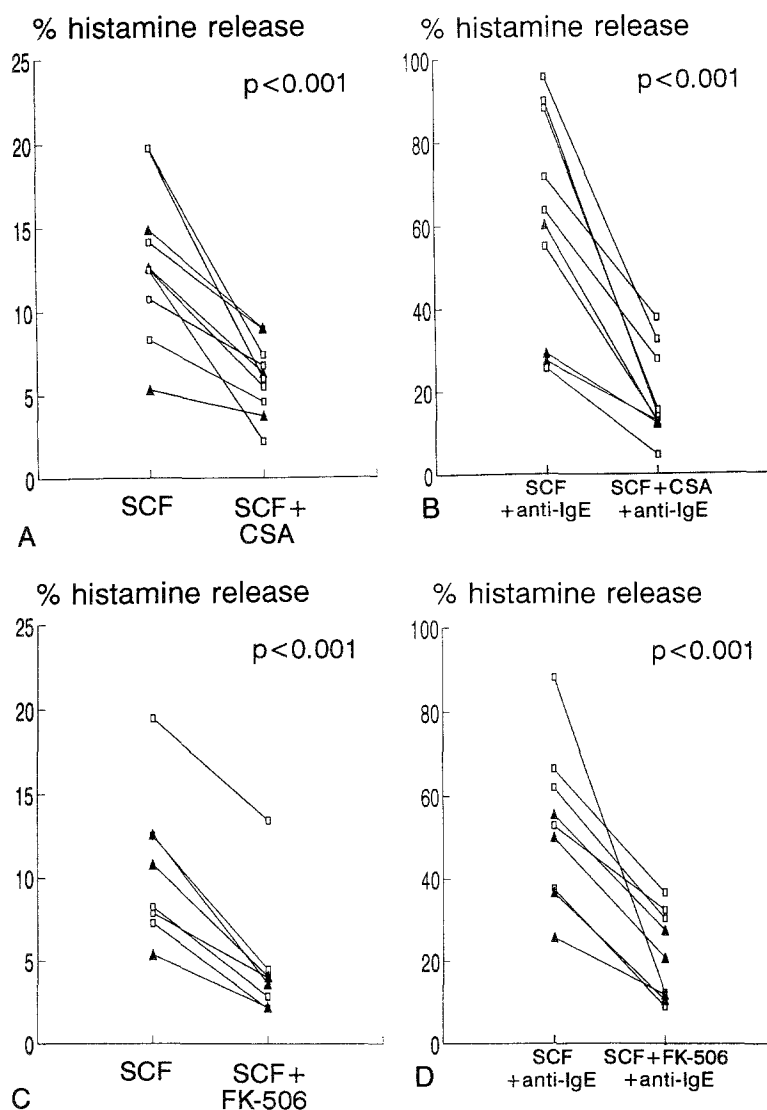
### Isolation of primary MCs

Human MCs were isolated from tissues by enzymatic treatment essentially as described in previous studies.<sup>12, 38-40</sup> Lung tissue was obtained (lobectomy) from 11 donors with bronchiogenic carcinoma (after informed consent had been obtained), according to the method described by Schulman et al.<sup>38</sup> Uterine MCs were obtained from 22 donors with myomatous uterus (hysterectomy), after informed consent was given, according to the method described by Massey et al.<sup>39</sup> In brief, tissue was put in Tyrode's buffer immediately after resection, chopped into small fragments, and washed extensively in  $Mg^{++}$  and  $Ca^{++}$  free Tyrode's buffer. Tissue fragments were incubated with collagenase (30 U/ml) at 37° C for 1 to 3 hours. Thereafter, dispersed cells were recovered by filtration through Nytex cloth (Rauscher Co., Vienna, Austria), washed three times in phosphate-buffered saline, and then examined for the percentage of MCs by toluidine blue staining. Tissue MCs were cultured in RPMI-1640 medium (Sera Lab, Crawley Down, U.K.) (with 10% fetal calf serum [FCS] at 37° C in 5%  $CO_2$ ) for at least 24 hours before being analyzed for histamine release. More than 80% of cells in short-term culture were viable according to dye exclusion criteria. The percentage of MCs ranged between 0.8% and 5.3% ( $2.9\% \pm 1.8\%$ ) in uterine cell suspensions and between 2.3% and 7.3% ( $4.5\% \pm 2.0\%$ ) in lung cell suspensions. The calculated amount of histamine was 0.8 to 2.6 pg ( $1.4 \pm 0.6$  pg) per MC in isolated uterine MCs and 0.6 to 3.1 pg ( $1.3 \pm 1.1$  pg) per MC in lung cell suspensions. The spontaneous release of histamine ranged between 1.3% and 6.9% in lung MCs and between 2.0% and 10% in uterine MCs.

The human MC leukemia cell line HMC-1<sup>41</sup> was kindly provided by J. H. Butterfield (Mayo Clinic, Rochester, Minn.). HMC-1 cells were cultured in Iscove's modified Dulbecco's medium supplemented with 10% FCS as described previously.<sup>16, 41</sup>

### Histamine release assay

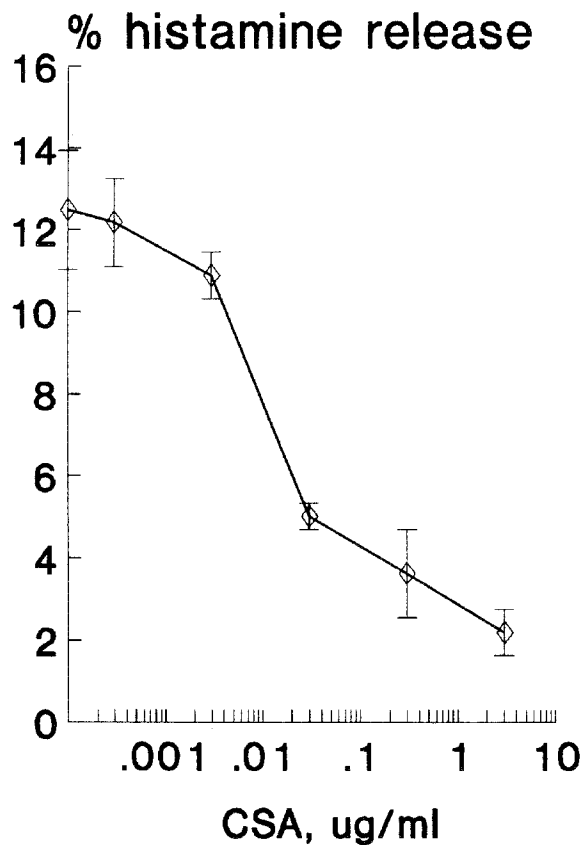
Histamine release assays were performed on tissue MCs according to published techniques.<sup>12, 42</sup> In typical experiments, MCs were first preincubated with CSA (0.0003 to 3  $\mu$ g/ml), FK-506, CSH, or control medium for 15 minutes (in case of IgE-dependent activation, MCs were also preincubated with IgE, 10  $\mu$ g/ml, for 3 hours). Then, cells were exposed to rhSCF (1 to 100 ng/ml) or control medium for 15 minutes and thereafter incubated with anti-IgE (0.01 to 10  $\mu$ g/ml) for another 30 minutes. When SCF was used as a single MC agonist, cells were exposed to SCF for 90 minutes. Cells were incubated in 96-well microtiter plates (Costar, Cambridge, Mass.). After incubation, the cells were centrifuged at 4° C, and the cell-free supernatants were recovered and analyzed for the presence of histamine. In selected experiments the time and sequence of addition of SCF and CSA or FK-506 were modified: cells were incubated first with SCF (15 minutes) and thereafter with CSA or FK-506 (15 minutes) or first with CSA (or



**FIG. 1. A and B,** Interactions between SCF and CSA on histamine release from human tissue MCs. MCs were isolated from tissues (uterus,  $n = 10$  [filled triangles]; lung,  $n = 4$  [open squares]) and prepared for histamine release as described in the text. **A,** Effect of CSA (3 µg/ml) preincubation on rhSCF-induced (10 ng/ml) histamine release from MCs (uterus,  $n = 7$ ; lung,  $n = 3$ ). **B,** Effects of CSA (3 µg/ml) on histamine release from MC (uterus,  $n = 7$ ; lung,  $n = 3$ ) induced by SCF (10 ng/ml, 15 minutes) plus anti-IgE (1 µg/ml, 30 minutes). Histamine release is expressed as percent of total histamine. **C and D,** Interactions between SCF and FK-506 on histamine release from human MCs (uterus,  $n = 6$  [filled triangles]; lung,  $n = 4$  [open squares]). **C,** Effects of FK-506 (3 µg/ml) on histamine release from MCs (uterus,  $n = 5$ ; lung,  $n = 3$ ) induced by rhSCF (10 ng/ml). **D,** Effect of FK-506 (3 µg/ml) on histamine release from MCs (uterus,  $n = 5$ ; lung,  $n = 4$ ) induced by rhSCF (10 ng/ml, 15 minutes) and anti-IgE (1 µg/ml, 30 minutes). Histamine release is expressed as percent of total histamine. No significant differences between lung and uterine MCs were found when the inhibitory effects of CSA and FK-506 were compared ( $p > 0.05$ ). The amount of histamine ranged between 0.8 and 2.6 pg ( $1.4 \pm 0.6$  pg) per MC in isolated uterine MCs and between 0.6 and 3.1 pg ( $1.3 \pm 1.1$  pg) per MC in lung cell suspensions. The spontaneous release of histamine amounted to 1.3% and 6.9% in lung MCs and between 2.0% and 10% in uterine MCs.

FK-506) and thereafter with SCF before anti-IgE stimulation. In time-course experiments, MCs were incubated with agonists or drugs for various lengths of time (up to 24 hours). Histamine release was calculated as

percent of total histamine. Total histamine was determined in cell lysates after freeze-thawing. Histamine was measured by a commercial radioimmunoassay (Immunotech) as described previously.<sup>12, 42</sup>



**FIG. 2.** Dose-dependent effect of CSA on SCF-induced histamine release from human tissue MCs. Isolated MCs were preincubated with various concentrations of CSA (as indicated) or control medium for 30 minutes. Then, cells were exposed to rhSCF (10 ng/ml) for 90 minutes, and (after centrifugation) the cell-free supernatants were analyzed for the presence of released histamine. Histamine release is expressed as percent of total histamine. Values represent the means  $\pm$  SD of quadruplicate determinations in one representative donor (uterine MCs).

#### Measurement of cyclic adenosine monophosphate (cAMP)

The cAMP levels were measured in HMC-1 cells after incubation with various concentrations of rhSCF, CSA (3  $\mu$ g/ml), CSH (3  $\mu$ g/ml), FK-506 (3  $\mu$ g/ml), or control medium. After various lengths of time (up to 240 minutes), intracellular cAMP levels were measured as described previously.<sup>43</sup> In brief, cells were washed in 50 mmol/L Tris-HCL buffer, pH 7.5, containing 0.5 mg/ml acetylsalicylic acid and 1 mmol/L aminophylline. Then, cells were homogenized by means of ultra-turrax and ultrasound, and the reaction was stopped by rapid centrifugation at 5000 g for 10 minutes (+4°C). The supernatants contained cAMP, which was measured by a commercially available cAMP radioimmunoassay (Amersham International, Buckinghamshire, U.K.), essentially according to the

manufacturer's instructions. Briefly, samples were incubated with iodine 125-labeled cAMP and rabbit anti-succinyl cAMP serum for 3 hours at 4°C. Thereafter, the antibody-bound cAMP was extracted by a donkey anti-rabbit serum, and coated to magnetizable polymer particles. Radioactivity was counted in a gamma counter for 1 minute. The sensitivity of the assay ranged from 0.25 to 16 fmol/ $\mu$ l.

#### Indirect immunofluorescence analyses

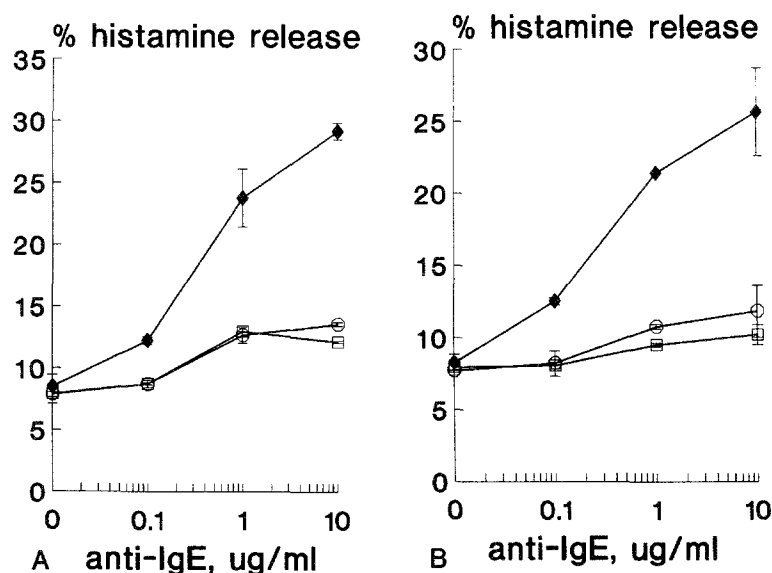
After incubation with various compounds (rhSCF [100 ng/ml], rhIL-4 [100 U/ml], CSA [3  $\mu$ g/ml], or FK-506 [3  $\mu$ g/ml]) for various time periods (30 minutes, 60 minutes, and 48 hours), HMC-1 cells were examined for expression of cell surface antigens (CD9, CD43, CD44, CD63, c-kit = CD117) by indirect immunofluorescence staining as described previously.<sup>40</sup> In brief, cells were incubated with mAbs (see above) for 30 minutes at 4°C, washed twice in phosphate-buffered saline, and conjugated with a "second-step" fluorescence-labeled goat F(ab')<sub>2</sub> IgG + IgM anti-mouse antibody (30 minutes at 4°C). Then, cells were analyzed by flow cytometry (FACS Scan, Becton Dickinson) as described.<sup>40</sup>

#### MC differentiation assay: LTC

Peripheral blood mononuclear cells of six healthy donors were obtained by Ficoll (Pharmacia, Uppsala, Sweden) gradient centrifugation. The MC differentiation assay was performed as described previously.<sup>5</sup> In brief, peripheral blood mononuclear cells were cultured at 37°C in 5% CO<sub>2</sub> in RPMI-1640 medium with 10% FCS, glutamine, and antibiotics in the presence or absence of rhSCF (100 ng/ml on day 0 and then 10 ng/ml every 2 weeks), CSA (0.0001 to 1.0  $\mu$ g/ml; drugs were added on day 0 and then every 2 weeks together with SCF), or FK-506 (0.0001 to 1.0  $\mu$ g/ml) in 24-well microtiter plates (Costar) for 42 days. Cells were fed in 2-week intervals and analyzed for total cell numbers (with a hemacytometer), differential cell counts, and tryptase levels. The cell morphology and the percentages of MCs were analyzed on cytopsin preparations after Giemsa staining. The absolute numbers of MCs were determined from total and differential cell counts. Because tryptase is an MC-specific marker, this enzyme was measured in cultured cells as a nonsubjective parameter of MC formation. Tryptase levels were determined in cell lysates after freeze-thawing by radioimmunoassay (Pharmacia) as described previously.<sup>5</sup> The detection limit for tryptase was 1 ng/ml. No cross-reactivity with histamine, heparin, rhSCF, rhIL-3, or rhIL-4 was found.

#### Statistical evaluation of data

Differences in mediator content and secretion were analyzed by standard tests including the paired Student's *t* test. Results were considered significantly different when the *p* value was less than 0.05.



**FIG. 3.** Effect of sequential treatment of human tissue MCs with rhSCF, CSA, or FK-506 on histamine release. Isolated tissue MCs were first preincubated with rhSCF (10 ng/ml, 15 minutes) and thereafter with CSA or FK-506 (3 µg/ml, 15 minutes) (open squares) or first with CSA or FK-506 and thereafter with rhSCF (open circles) before challenge with various concentrations of anti-IgE (as indicated). For technical details see text. **A**, Effect of CSA (3 µg/ml) on MCs. **B**, Effect of FK-506 (3 µg/ml) on MCs. The response of MCs to anti-IgE after preincubation with SCF in the absence of drugs (control release) is indicated by the upper graphs (filled diamonds). Results are given as percentage of total histamine. Values represent the means  $\pm$  SD of quadruplicate determinations from one donor (uterine MCs). Spontaneous release of histamine was 7.5% of total histamine.

## RESULTS

### Effects of CSA and FK-506 on SCF-dependent histamine release from human tissue MCs

According to previous observations<sup>12</sup> rhSCF (1 to 100 ng/ml, 90-minute incubation) induced release of histamine from human MCs (SCF: 22.8%  $\pm$  13.4% vs control: 6.2%  $\pm$  3.9%,  $p < 0.001$ ). The effect of rhSCF on histamine release was dose- and time-dependent. Other cytokines (including rhIL-3 and rhIL-4) did not induce mediator release from human MCs. Preincubation of MCs with CSA (3 µg/ml) resulted in inhibition of mediator release induced by either rhSCF, 10 ng/ml (70.3%  $\pm$  20.6% inhibition,  $p < 0.001$ ) (Fig. 1, *A*), or anti-IgE (76.7%  $\pm$  21.9% inhibition,  $p < 0.001$ ). CSA also inhibited histamine release induced by anti-IgE plus rhSCF (control: 6.9%  $\pm$  3.5%, anti-IgE + SCF: 61.9%  $\pm$  27.4% vs anti-IgE + SCF + CSA: 17.5%  $\pm$  11.4% histamine release = 77.4%  $\pm$  13.9% inhibition,  $p < 0.001$ ) (Fig. 1, *B*). Similar results (with lower inhibitory concentration of 50% [ $IC_{50}$ ] values) were obtained for FK-506. Again, the drug produced inhibition of direct histamine release (SCF-induced release: 82.0%  $\pm$  18.9%

inhibition,  $p < 0.001$ ; anti-IgE-induced release: 71.5%  $\pm$  16.7% inhibition,  $p < 0.001$ ) (see also Fig. 1, *C*) and also inhibited histamine release induced by SCF plus anti-IgE (70.0%  $\pm$  17.3% inhibition,  $p < 0.001$ ) (Fig. 1, *D*). The effects of CSA and FK-506 on histamine release were found to be dose-dependent ( $IC_{50}$  [CSA]: 1 to 10 ng/ml;  $IC_{50}$  [FK-506]: 0.3 to 3 ng/ml, for MC activation induced by either SCF or anti-IgE) (see also Fig. 2). About three to 10 times higher  $IC_{50}$  values were obtained for MCs preincubated with rhSCF for 15 minutes before challenge with anti-IgE, compared with anti-IgE alone. No significant differences in CSA- or FK-506-induced inhibition of SCF-dependent secretion were found when data from uterine MCs and lung MCs were compared ( $p > 0.05$ , Fig 1). CSH, an inactive CSA analog (lacking high-affinity for cyclophilins), did not inhibit SCF-dependent or SCF-independent activation of MCs ( $IC_{50} > 3$  µg/ml).

To determine whether the time and sequence of addition of SCF and CSA (or FK-506) would have an influence on the effects of the drugs on mediator release, MCs were first incubated with rhSCF for 15 minutes and thereafter with drugs (15 minutes), or MCs were first preincubated with

**TABLE 1.** Effects of CSA and FK-506 on SCF-dependent formation of MCs and tryptase in LTC

<b>A. Effect of CSA</b>												
	Donor 1			Donor 2			Donor 3			Donor 4		
	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)
co	1.17	0	—	<1	—	—	1.42	0	—	1.39	—	—
SCF	17.55	3,840	4.6	15.33	4,500	3.4	2.23	4,800	0.5	3.86	9,620	0.4
CSA 1	21.47	8,600	2.5	19.34	7,820	2.5	7.28	9,800	0.7	45.42	44,550	1.0
CSA 0.1	25.29	6,240	4.1	17.17	4,000	4.3	5.86	19,760	0.3	20.48	19,440	1.1
CSA 0.01	47.99	15,640	3.1	30.72	8,400	3.7	11.42	25,500	0.4	2.56	10,240	0.3
CSA 0.001	28.29	3,400	8.3	37.92	11,590	3.3	8.3	18,200	0.5	—	—	—
CSA 0.0001	24.9	3,600	6.9	16.25	8,000	2.0	—	—	—	—	—	—

<b>B. Effect of FK-506</b>												
	Donor 1			Donor 2			Donor 3			Donor 4		
	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)
co	1.17	0	—	<1	—	—	1.42	—	—	1.39	—	—
SCF	17.55	3,840	4.6	15.33	4,500	3.4	2.23	4,800	0.5	3.86	9,620	0.4
FK 1	1.30	—	—	25.79	6,300	4.1	9.80	5,500	1.9	12.56	20,250	0.6
FK 0.1	10.80	10,250	1.1	12.88	10,000	1.2	13.34	13,320	1.0	24.25	24,000	1
FK 0.01	21.17	13,000	1.6	34.99	16,000	2.2	10.17	12,600	0.8	34.97	32,000	1.1
FK 0.001	40.41	15,500	2.6	45.22	30,100	1.5	13.50	12,800	1.1	—	—	—
FK 0.0001	35.51	9,660	3.7	18.50	8,100	2.3	—	—	—	—	—	—

MCs were cultured from peripheral blood mononuclear cells by using rhSCF as described in the text. Part A shows the effects of CSA (various concentrations as indicated) on SCF-dependent formation of MCs in six donors, and part B shows the effects of FK-506 (various concentrations) in four donors. Cultures were harvested on day 42. The numbers of cultured MCs (per milliliter) on day 42 were calculated on the basis of the total number of cells (assessed by a hemocytometer) and the percentage counts of MCs on Giemsa stained slides. MC tryptase (*Tryp*) was measured as an objective parameter of MC differentiation. The amounts of tryptase per MC are also shown. Results in each donor (experiment) represent the mean of duplicate cultures. The mean  $\pm$  SEM values from all donors are also provided.

CSA or FK-506 and thereafter with rhSCF before challenge with anti-IgE. The results of these experiments showed that both CSA and FK-506 block histamine release induced by anti-IgE, even when MCs had been primed with rhSCF (Fig. 3, A and B). Spontaneous release of histamine (without addition of any factor) was below 11% in all experiments ( $5.5\% \pm 3.6\%$ ).

#### Effects of CSA and FK-506 on SCF-dependent formation of cAMP in human MCs

Activation of MCs (or basophils) is often associated with changes in intracellular cAMP levels. In this study rhSCF was found to upregulate cellular cAMP levels in HMC-1 cells by up to 3.2 times (SCF, 100 ng/ml:  $230\% \pm 40\%$  of control after 120 minutes). The effect of SCF on cellular cAMP formation in HMC-1 cells was found to be time-dependent (maximum effect [150% to 250% of control] observed after 60 to 120 minutes). Both CSA and FK-506 failed to downregulate but in-

duced upregulation of cAMP levels in HMC-1 cells. Moreover, both drugs failed to modulate (i.e., upregulate or inhibit) SCF-dependent increases of cAMP in HMC-1 cells ( $p > 0.05$ ).

#### Effects of CSA and FK-506 on expression of SCF receptor in HMC-1 cells

Activation of MCs by SCF is mediated through the c-kit receptor (CD117). Therefore we attempted to determine whether CSA or FK-506 would interact with expression of c-kit. For this purpose, the human MC line HMC-1 was incubated with CSA (3  $\mu$ g/ml), CSH (3  $\mu$ g/ml), FK-506 (3  $\mu$ g/ml), rhIL-4 (100 U/ml), or rhSCF (100 ng/ml) for up to 48 hours and analyzed for expression of CD117/c-kit by using mAb YB5.B8. Over the time range tested, the drugs failed to induce any changes in expression of CD117/c-kit or expression of other cell surface antigens including CD9 (p24), CD43 (leukosialin), CD44 (Pgp-1), or CD63 (MC activation antigen), whereas rhIL-4 induced downregulation of c-kit as reported.<sup>16</sup>

Donor 5			Donor 6			Mean $\pm$ SEM of all donors		
Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)	Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)
1.58	0	—	6.1	—	—	2.33 $\pm$ 1.88	0 $\pm$ 0	—
24.54	11,400	2.2	53.3	18,200	2.9	19.47 $\pm$ 16.99	8,727 $\pm$ 5,080	2.33 $\pm$ 1.51
29.99	15,000	2.0	277.3	30,000	9.2	66.80 $\pm$ 94.84	21,590 $\pm$ 13,777	2.98 $\pm$ 2.86
9.69	9,750	1.0	169.7	15,000	11.3	41.37 $\pm$ 57.75	12,365 $\pm$ 6,137	3.68 $\pm$ 3.74
3.47	9,120	0.4	155.0	16,100	9.6	41.86 $\pm$ 53.08	14,167 $\pm$ 5,895	2.91 $\pm$ 3.29
—	—	—	—	—	—	24.84 $\pm$ 12.34	11,063 $\pm$ 6,054	4.03 $\pm$ 3.23
—	—	—	—	—	—	20.58 $\pm$ 4.32	5,800 $\pm$ 2,200	4.45 $\pm$ 2.45

Mean $\pm$ SEM of all donors		
Tryp (ng/ml)	MCs/ ml	Tryp (pg/MC)
1.33 $\pm$ 0.11	0 $\pm$ 0	—
9.74 $\pm$ 7.77	5,690 $\pm$ 2,295	2.23 $\pm$ 1.83
12.36 $\pm$ 8.79	10,683 $\pm$ 6,773	2.16 $\pm$ 1.45
15.32 $\pm$ 5.25	14,393 $\pm$ 5,699	1.08 $\pm$ 0.08
25.33 $\pm$ 10.41	18,400 $\pm$ 7,961	1.43 $\pm$ 0.53
33.04 $\pm$ 13.96	19,467 $\pm$ 7,599	1.73 $\pm$ 0.63
27.01 $\pm$ 8.51	8,880 $\pm$ 780	3.00 $\pm$ 0.70

### Effects of CSA and FK-506 on SCF-dependent growth of human MCs

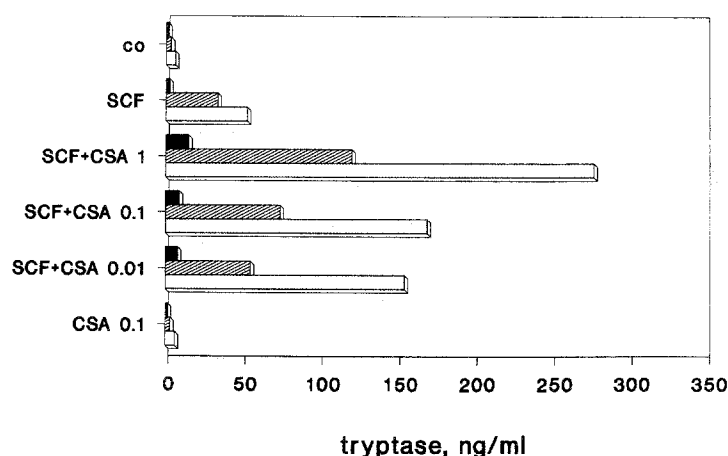
Recently, we were able to show that rhSCF induces differentiation of human MCs from their circulating progenitor cells in LTC.<sup>5</sup> In this study the effects of CSA and FK-506 on SCF-dependent differentiation of human MCs (input of cells on day 0:  $0.5 \times 10^6$ /ml) were analyzed ( $n = 6$ ). In these cultures, rhSCF induced formation of MCs (MC numbers on day 42: control,  $<1$  vs rhSCF, 100 ng/ml,  $8727 \pm 5080$  MC per milliliter), as well as synthesis of MC tryptase (total tryptase levels on day 42: control,  $2.3 \pm 1.9$  ng/ml vs rhSCF, 100 ng/ml,  $19.5 \pm 17.0$  ng/ml). Unexpectedly, both CSA and FK-506 were found to upregulate SCF-dependent formation of MCs in a dose-dependent way (Table I). Addition of CSA, 1  $\mu$ g/ml, or FK-506, 0.001  $\mu$ g/ml, (optimal concentrations of drugs) produced a 2.5- and 3.4-fold increase in the total number of MCs on day 42, respectively. Synthesis of cellular tryptase (an MC marker enzyme) was measured as an MC parameter in LTC. CSA (1  $\mu$ g/ml) induced a 3.6-fold increase, and FK-506 (0.001  $\mu$ g/ml = optimal concentration) induced a 3.4-fold increase in total cellular tryptase levels compared with rhSCF alone. The effects of

the drugs on MC differentiation and synthesis of cellular tryptase were found to be dose- and time-dependent with optimal stimulation observed with 0.01 to 1  $\mu$ g/ml CSA and 0.001 to 0.01  $\mu$ g/ml FK-506 (Table I). Fig. 4 shows the dose- and time-dependent effect of CSA on SCF-dependent MC growth in one donor. A summary of results (all six donors) is shown in Table I.

A good correlation between MC numbers and total tryptase levels was found in our LTCs. In two of six donors, the tryptase levels per MC were found to be higher (about two- to threefold) in cultures containing CSA or FK-506 as compared with rhSCF alone, whereas the overall increase (all donors) showed no significant difference in "tryptase per MC" levels, when the different cultures (presence or absence of drugs) were compared ( $p > 0.05$ ) (see Table I). In the absence of SCF, MCs could not be detected in LTC under any culture condition.

### DISCUSSION

Recent data suggest that SCF is involved in the regulation of differentiation and function of human MCs.<sup>5, 6, 10-12</sup> In this study the in vitro effects of CSA and FK-506 on SCF-dependent growth and function of human MCs were analyzed. The



**FIG. 4.** Dose- and time-dependent effect of CSA on SCF-induced formation of MC tryptase in a peripheral blood LTC system. Peripheral blood mononuclear cells were obtained by Ficoll gradient centrifugation and cultured in complete medium with 10% FCS (at 37° C in 5% CO<sub>2</sub>) in the presence or absence of rhSCF (100 ng/ml on day 0, 10 ng/ml every 2 weeks) or rhSCF + CSA (various concentrations as indicated) in 24-well microtiter plates for 42 days. Cells were fed in 2-week intervals and analyzed for total cell counts and cellular tryptase levels on days 14 (*black bars*), 28 (*hatched bars*), and 42 (*stippled bars*). Results from one donor (donor 6 in Table I) are shown.

results of this study show that both CSA and FK-506 inhibit SCF-dependent activation and mediator release from tissue MCs but promote SCF-dependent formation of MCs in an LTC system.

SCF has recently been described as a selective and potent activation factor for human MCs.<sup>10-12</sup> In particular, rhSCF co-operates with anti-IgE in inducing mediator release from MCs. Furthermore, rhSCF caused direct release of proinflammatory mediators from MCs.<sup>11, 12</sup> These effects of rhSCF were confirmed in this study by using lung and uterine MCs. It is important to know that the direct effect of rhSCF occurs (in all tissue MCs) after a prolonged time of incubation (after 45 minutes), whereas IgE-dependent histamine secretion in MCs can already be seen after 15 minutes of incubation.

CSA and FK-506 are two potent immunosuppressants with multifunctional antiinflammatory activities.<sup>19-27</sup> Both drugs inhibit immune cell activation and proliferation.<sup>19, 23-26, 35-37</sup> In this study CSA and FK-506 were found to inhibit histamine release induced by rhSCF (90 minutes), by anti-IgE (30 minutes), or by rhSCF + anti-IgE (15 minutes plus 30 minutes, respectively). The inhibitory effects of CSA and FK-506 on MC functions were found to be dose-dependent. The IC<sub>50</sub> values were higher for MCs preincubated with rhSCF and thereafter stimulated with anti-IgE, compared with anti-IgE stimulation alone. This might be of clinical relevance because tissue MCs are well known to express SCF receptors *in situ*<sup>14</sup> and are probably

less sensitive to the inhibitory effects of CSA and FK-506 when exposed to (endogenous or exogenous) SCF *in vivo*. On the other hand, high concentrations of CSA and FK-506 (1 to 10 µg/ml) may (almost) completely block MC release induced by IgE receptor activation, even when MCs are primed by SCF (15 minutes). Whether the beneficial effects of CSA in patients with allergic asthma,<sup>45</sup> autoimmune diseases,<sup>20-22</sup> or graft versus host disease or graft rejection (prophylaxis) are due to MC (and/or basophil) deactivation for mediator release remains to be elucidated.

Recently, CSA and FK-506 have been shown to deactivate basophils for mediator release.<sup>29, 32, 34</sup> IL-3 is a potent activator of blood basophils<sup>42, 46-48</sup> but did not induce or promote mediator secretion by human MCs.<sup>49</sup> IL-3 has also been described to counteract the inhibitory effects of CSA and FK-506 on human basophils.<sup>29</sup> Thus CSA and FK-506 may have similar effects on MCs and basophils in terms of cytokine-dependent cell activation for mediator release, although the principal activator molecules are quite different (SCF for MCs and IL-3 for basophils).

In the past few years great efforts have been undertaken to elucidate the mechanisms of action of CSA and FK-506. CSA and FK-506 apparently act on their target cells through a family of specific receptors (i.e., cyclophilins) exhibiting rotamase activity.<sup>50-55</sup> The existence of such binding sites has been confirmed for human MCs and human basophils.<sup>29-32</sup> In this study the involvement of cyclo-



philins in CSA-induced inhibition of SCF-dependent histamine release seems likely because the cyclophilin-inactive CSA analog CSH showed no effect. We also asked whether the deactivation by CSA/FK-506 was associated with changes in expression of MC antigens. Apparently, SCF acts on human MCs through the c-kit receptor (CD117) and upregulates formation of cAMP in HMC-1. However, CSA and FK-506 failed to modulate expression of SCF binding sites or expression of other MC antigens and failed to inhibit SCF-dependent or SCF-independent expression of cAMP in HMC-1 cells. Similar results were obtained with normal MCs (unpublished). These observations suggest that CSA and FK-506 down-regulate MC histamine release induced by SCF distal to the c-kit receptor (CD117) and probably not through modulation of cAMP levels.

Recent data have shown that SCF is a specific growth factor for human MCs.<sup>5</sup> The observation that CSA and FK-506 do not inhibit but even promote SCF-dependent formation of human MCs in LTC was unexpected because the drugs reportedly inhibit (cytokine-dependent) growth of human T cells<sup>23,24</sup> and because so far no experimental promotor of SCF-dependent growth of human MCs has been described. Rather, treatment with corticosteroids produced inhibition of MC growth in fetal liver cell cultures,<sup>56</sup> and the cytokines IL-4 and IL-3 (both mouse MC growth factors), as well as tumor necrosis factor, were found to inhibit SCF-dependent differentiation of human MCs.<sup>57,58</sup> Therefore it might well be that the effects of CSA and FK-506 on MC differentiation were in part due to inhibition of production of cytokines (IL-3, IL-4, or tumor necrosis factor) in T cells or maturing MCs in LTCs. Alternatively, CSA and FK-506 inhibited continuous MC depletion in LTCs by preventing degranulation and subsequent loss of cell viability. A third possibility would be that CSA and FK-506 exerted a direct (growth-promoting) effect on the MC progenitor cells.

In conclusion, this study provides evidence that both CSA and FK-506 inhibit SCF-dependent activation and mediator secretion in mature human tissue MCs but do not inhibit SCF-dependent differentiation of human MCs in vitro.

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