



# Effects of Bucillamine on Antigen-Presenting Cells in Experimental Autoimmune Uveitis in Rats

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**Abstract:** Bucillamine, an anti-rheumatic drug, was compared with cyclosporine (CYA) in its effects on the antigen-presentation activity of antigen-presenting cells (APCs) and the antigen-specific proliferation of T cells from S-antigen-immunized rats. In vitro assay showed that bucillamine did not affect the proliferative response of T cells, but suppressed the antigen-presenting activity of APCs, such as macrophages and retinal pigment epithelial cells. On the other hand, CYA suppressed both T cell proliferation and the antigen-presenting activity of macrophages. However, the doses of CYA required to produce significant suppression of antigen presentation were higher than those needed to inhibit T cell proliferation. Daily systemic administration of bucillamine for 14 days after immunizing rats with S-antigen suppressed the intensity of experimental autoimmune uveitis (EAU) and the antigen-presenting activity of macrophages in treated rats, but not the antigen-specific proliferation of the T cells. EAU intensity was completely suppressed by CYA for 14 days post-immunization, and antigen-specific proliferation of T cells was suppressed, but the antigen-presenting activity of macrophages was not affected. These results suggested that the suppressive effects of bucillamine on the antigen-presenting activity of APCs contributed to its suppressive effect on EAU; whereas, the suppressive effects of CYA on EAU resulted principally from its suppression of the T-cell function. **Jpn J Ophthalmol 1997;41:388-395** © 1997 Japanese Ophthalmological Society

**Key Words:** Antigen presentation, bucillamine, cyclosporine, experimental autoimmune uveitis, rats, retinal pigment epithelial cells.

## Introduction

Bucillamine [*N*-(2-mercapto-2-methylpropionyl)-*L*-cysteine] (Figure 1), is an anti-rheumatic drug that has been reported to be effective in rheumatoid arthritis.<sup>1-5</sup> This agent had prophylactic and therapeutic effects on adjuvant-induced arthritis in rats.<sup>6</sup> Although the precise action mechanisms of bucillamine are not yet known, there are several lines of evidence which indicate that bucillamine affects the immune system.<sup>6-10</sup>

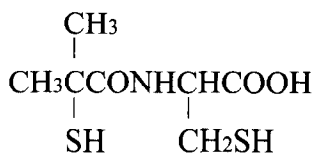
We previously reported that bucillamine inhibited the development of S-antigen-induced experimental

autoimmune uveitis (EAU) in rats, and that combination therapy with bucillamine and a low dose of cyclosporine (CYA) was more effective than single drug therapy with either.<sup>11</sup> Furthermore, we assayed the proliferative response of spleen cells obtained from the S-antigen-immunized and drug-treated rats. The antigen-specific proliferative response of spleen cells obtained from bucillamine-treated rats was significantly suppressed, and the suppression was significantly overcome by removing adherent cells from the culture.<sup>11</sup> These results suggest that the inhibitory effect of bucillamine on lymphocyte proliferation might, in part, be caused by its effects on the antigen-presenting cells (APCs).

The present study was, therefore, aimed at analyzing the in vitro and in vivo effects of bucillamine on the antigen-presenting activity of APCs and the proliferative response of T cells obtained from S-antigen-immunized rats.

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**Figure 1.** Molecular structure of bucillamine. Molecular weight: 322.3.

## Materials and Methods

### Animals

Lewis rats were purchased from Charles River Japan (Atsugi, Kanagawa). Brown Norway rats were purchased from Seiwa Experimental Animals Ltd. (Fukuoka). All animal procedures were conducted in accordance with the ARVO Resolution on the Use of Animals in Research.

### Drugs

Bucillamine was supplied by Santen Pharmaceutical Co., Ltd. (Osaka). CYA was supplied by Sandoz Ltd. (Basel, Switzerland).

**In vitro assay.** Bucillamine was dissolved in RPMI-1640 medium (Gibco, Grand Island, NY, USA). CYA was dissolved in ethanol at a concentration of 50 mg/mL and used to prepare various concentrations of the drug in RPMI-1640 medium. The final concentration of ethanol never exceeded 0.02% of the medium. A preliminary experiment confirmed that the concentration of ethanol did not affect the proliferative response and antibody production of spleen cells.

**In vivo assay.** Bucillamine was suspended in 0.5% tragacanth solution and administered orally. CYA was dissolved in pure olive oil and administered by intramuscular injection at the hindfoot. Rats were treated with either bucillamine (20 mg/kg per day or 200 mg/kg per day) or CYA (10 mg/kg per day). Control rats received only 0.5% tragacanth solution. The therapy was carried out once a day for 14 days post immunization.

### Antigen and Immunization

Retinal soluble antigen (S-antigen) was prepared from fresh bovine retinas according to the method of Dorey et al.<sup>12</sup> The antigen was emulsified (1:1) with complete Freund's adjuvant (Difco, Detroit, MI, USA), containing *Mycobacterium tuberculosis H37Ra* (Difco) at a concentration of 2.0 mg/mL.

**In vitro assay.** A total of 0.1 mL/rat of the emulsion containing 50 µg of S-antigen was injected into one

footpad of male Lewis or Brown Norway rats (7–8 weeks old). Four weeks after the immunization, the animals were given an S-antigen booster of 50 µg/rat.

**In vivo assay.** A total of 0.1 mL/rat of the emulsion containing 30 µg of S-antigen was injected into one footpad of male Lewis rats (7–8 weeks old).

### Preparation of T Cells and Macrophages

The spleen cells were isolated from the rats on day 14 after the immunization with S-antigen, as described previously.<sup>11</sup> The spleen cells were suspended with RPMI-5%FCS, consisting of RPMI-1640 medium, 5% heat-inactivated fetal calf serum (FCS) (Gibco), 100 U/mL penicillin (Gibco) and 100 µg/mL streptomycin (Gibco).

**Macrophage fraction (adherent cells).** Ten milliliters of the spleen cell suspension ( $1 \times 10^6$  cells/mL) was incubated in a plastic petri dish (100 × 15 mm, Costar, Cambridge, MA, USA) for 2 hours at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. Non-adherent cells were removed by washing three times with RPMI-5%FCS and used for further preparation of the T cell fraction. Adherent cells were harvested and suspended with RPMI-5%FCS containing anti-rat CD2 monoclonal antibody (1:1000) (MRC OX-34, Serotec, Oxford, UK), and then incubated for 45 minutes at 4°C. The reaction mixture was centrifuged at  $200 \times g$  for 5 minutes at 4°C and the pellet was resuspended in RPMI-5%FCS containing rabbit complement (1:20; Serotec) for 45 minutes under culture conditions. The cell suspension was washed three times with RPMI-5%FCS and used as the macrophage fraction. The cell suspension was examined by flow cytometry after staining the cells with anti-rat Ia antigen monoclonal antibody (MRC OX 17, Serotec). Approximately 75% of the cells were Ia antigen-positive cells. A morphological examination using a phase contrast microscope revealed that living cells (trypan blue-unstained cells) contained 98% adherent cells.

**T-cell fraction (nonadherent cells).** A T-cell fraction was prepared using a technique described by Julius et al.<sup>13</sup> Briefly, sterilized nylon wool-packed columns were preincubated with RPMI-5%FCS for 1 hour under culture conditions. One milliliter of the plastic-nonadherent cell suspension ( $1 \times 10^8$  cells/mL) was loaded onto the columns and incubated for 45 minutes under culture conditions. Then the nylon wool columns were washed with warmed RPMI-5%FCS, and the initial 5–15 mL of effluent was collected and used as T-cell fraction (nonadherent cells). The fraction was examined by staining with anti-rat

CD2 monoclonal antibody and analyzing by flow cytometry. Approximately 96% of the cells were CD2-positive cells. A morphological examination using a phase contrast microscope revealed that only 0.02% of cells in the fraction adhered to the plastic dish.

### *Preparation of Retinal Pigment Epithelial (RPE) Cells*

RPE cells were prepared from naive Brown Norway rats according to the method of Chang et al.<sup>14</sup> Briefly, eyes were enucleated from the rats (7–8 days old) and washed 3 times with Dulbecco's modification Eagle's medium (DMEM; Gibco) containing 100 U/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin B (Gibco), and 2 mmol/L L-glutamine (Gibco). The eyes were incubated for 30 minutes at 37°C in DMEM supplied with 2% Dispase (grade II, Boehringer Mannheim, Indianapolis, IN, USA), and dissected under a dissecting microscope. The retina with the adherent RPE sheet was lifted from the choroid and sclera and the RPE sheets were separated from the retinas with fine forceps. Pooled RPE sheets were rinsed with calcium- and magnesium-free Hank's balanced salt solution (HBSS; Gibco). The RPE sheets were treated with HBSS containing 0.1% trypsin (Sigma Chemical, St. Louis, MO, USA) for 3 minutes under culture conditions and then dissociated into single cells by gentle pipetting. The trypsinization was terminated by addition of DMEM containing an excess of 10% FCS.

### *Flow Cytometry Analysis*

The T-cell fraction or macrophage fraction ( $5 \times 10^5$  cells) was incubated with 5 µg of anti-rat CD2 monoclonal antibody or anti-rat Ia antigen monoclonal antibody for 45 minutes at 4°C. The reaction mixture was washed three times and the cells were incubated with 50 µL of FITC-conjugated rabbit anti-mouse IgG monoclonal antibody (1:100) (Sero-tec) for 45 minutes at 4°C. A mouse-derived monoclonal antibody of the same class, with no specificity for rat T cells and macrophages, was used as a control. Each sample was analyzed by flow cytometry.

## **Assay for Drug Effects on <sup>3</sup>H-thymidine Uptake by T Cells**

### *In Vitro Treatment*

**Antigen-presenting activity of macrophages.** *Drug treatment of macrophages.* The macrophage fraction ( $1 \times 10^6$  cells/mL) from S-antigen-immunized Lewis rats was cultured in RPMI-5%FCS in the

presence of buccillamine for 0–2 hours or CYA for 2 hours, and S-antigen (20 µg/mL) was added to the culture. The macrophage suspension was further cultured for 2 hours; then washed three times and suspended in RPMI-10%FCS supplemented with  $1 \times 10^{-5}$  mol/L of 2-mercaptoethanol, 100 U/mL penicillin and 100 µg/mL streptomycin.

**Drug pre-treatment of macrophages.** The macrophage fraction ( $1 \times 10^6$  cells/mL) from S-antigen-immunized rats was cultured in RPMI-5%FCS in the presence of buccillamine ( $10^{-8}$ – $10^{-4}$  mol/L) for 4 hours, then washed three times. The macrophages were suspended in medium containing S-antigen (20 µg/mL) and incubated for 2 hours; then washed three times and suspended in RPMI-10%FCS.

**Drug post-treatment of macrophages.** The macrophage fraction ( $1 \times 10^6$  cells/mL) from S-antigen-immunized rats was cultured in RPMI-5%FCS in the presence of S-antigen (20 µg/mL) for 2 hours; then washed three times. The macrophages were suspended in medium containing buccillamine ( $10^{-8}$ – $10^{-4}$  mol/L) and incubated for 4 hours; then washed three times and suspended in RPMI-10%FCS.

The drug- and antigen-treated macrophages ( $2.5 \times 10^5$  cells/mL) were co-cultured for 4 days with T cells ( $2.5 \times 10^6$  cells/mL) obtained from S-antigen-immunized rats. The cultures were primed with <sup>3</sup>H-thymidine (TdR) (0.5 µCi/well) for the last 16 hours of culture. Incorporated <sup>3</sup>H-TdR was assayed by a liquid scintillation counter.

### **Antigen-specific proliferative response of T cells.**

The T-cell fraction ( $1 \times 10^6$  cells/mL) from S-antigen-immunized Lewis rats was cultured in RPMI-5%FCS together with buccillamine ( $10^{-8}$ – $10^{-4}$  mol/L) or CYA ( $10^{-8}$ – $10^{-4}$  mol/L) for 4 or 24 hours; then washed three times and suspended in RPMI-10%FCS. The macrophage fraction ( $1 \times 10^6$  cells/mL) was cultured in the presence of S-antigen (20 µg/mL) for 2 hours, washed three times, and resuspended in RPMI-10%FCS (S-antigen-pulsed macrophages). The drug-treated T cells ( $2.5 \times 10^6$  cells/mL) and the S-antigen-pulsed macrophages ( $2.5 \times 10^5$  cells/mL) were co-cultured in a 96-well flat-bottomed culture plate in RPMI-10%FCS for 4 days. The proliferative response of T cells was assayed by the uptake of <sup>3</sup>H-TdR as described above.

**Pinocytotic activity of macrophages.** The macrophage fraction from S-antigen-immunized rats was cultured in RPMI-5%FCS in the presence of buccillamine for 4 hours at 37°C, and FITC-conjugated-S-antigen (100 µg/mL) was added to the culture. The macro-

phage suspension was further cultured for 30 minutes at 37°C, then washed three times and suspended in PBS containing 1% formaldehyde. As a negative control, the bucillamine-treated macrophages were cultured in FITC-conjugated-S-antigen-containing medium for 30 minutes at 10°C to minimize the pinocytotic activity. Treated macrophages were analyzed with a flow cytometry (Epics-Profile, Coulter, Hialeah, FL, USA). The pinocytotic activity of macrophages was expressed as intensity of intracellular fluorescence.

**Antigen-presenting activity of RPE cells.** After RPE cells ( $5 \times 10^5$  cells/mL) from Brown Norway rats were cultured in DMEM supplemented with 10% FCS for 1 week until confluent, the cells were treated with bucillamine for 4 hours and pulsed with S-antigen (20  $\mu$ g/mL) for the last 2 hours. After washing the culture, the RPE cells in DMEM-10%FCS were cultured in the presence of mitomycin C (100  $\mu$ g/mL) for 1 hour to terminate cell proliferation, and washed three times with RPMI-10%FCS. The RPE cells were then co-cultured for 4 days with T cells ( $5 \times 10^6$  cells/mL) obtained from S-antigen-immunized Brown Norway rats in RPMI-10%FCS containing 1  $\mu$ g/mL indomethacin. The proliferative response of T cells was assayed by the uptake of  $^3$ H-TdR.

#### *In Vivo Treatment*

**Antigen-presenting activity of macrophages.** The macrophage fraction ( $1 \times 10^6$  cells/mL) from the drug-treated or untreated (control) rats was cultured in RPMI-5%FCS supplemented with 20  $\mu$ g/mL of S-antigen for 2 hours. The macrophage fraction was washed three times and suspended in RPMI-

10%FCS. The S-antigen-pulsed macrophages ( $2.5 \times 10^5$  cells/mL) and T cells obtained from control rats ( $2.5 \times 10^6$  cells/mL) were co-cultured in RPMI-10%FCS for 4 days, and the proliferative response of T cells was assayed by the uptake of  $^3$ H-TdR.

**Proliferative response of T cells.** The T-cell fraction from the drug-treated or control rats was suspended in RPMI-10%FCS ( $5 \times 10^6$  cells/mL). The macrophage fraction from control rats ( $1 \times 10^6$  cells/mL) was pulsed with S-antigen (20  $\mu$ g/mL) for 2 hours, washed three times, and suspended in RPMI-10%FCS. The T cells ( $2.5 \times 10^6$  cells/mL) and the macrophages ( $2.5 \times 10^5$  cells/mL) were co-cultured in RPMI-10%FCS for 4 days, and the proliferative response of T cells was assayed by the update of  $^3$ H-TdR.

## Results

### *In Vitro Treatment*

**Drug effects on antigen-presenting activity of macrophages.** The  $^3$ H-TdR uptake by the culture consisting of S-antigen-sensitized T-cell fraction and drug-treated macrophage fraction was suppressed by treatment of the macrophage fraction with bucillamine ( $10^{-5}$  mol/L), the effects were dependent on the preincubation time of the macrophage fraction with the drug (Table 1). The highest inhibition was achieved by preincubation with bucillamine for 120 minutes. A dose-response study was, therefore, performed with 120 minute-preincubation and various concentrations of bucillamine (Figure 2A). The  $^3$ H-TdR uptake by the culture of T-cell and macrophage fractions was inhibited by bucillamine pretreatment

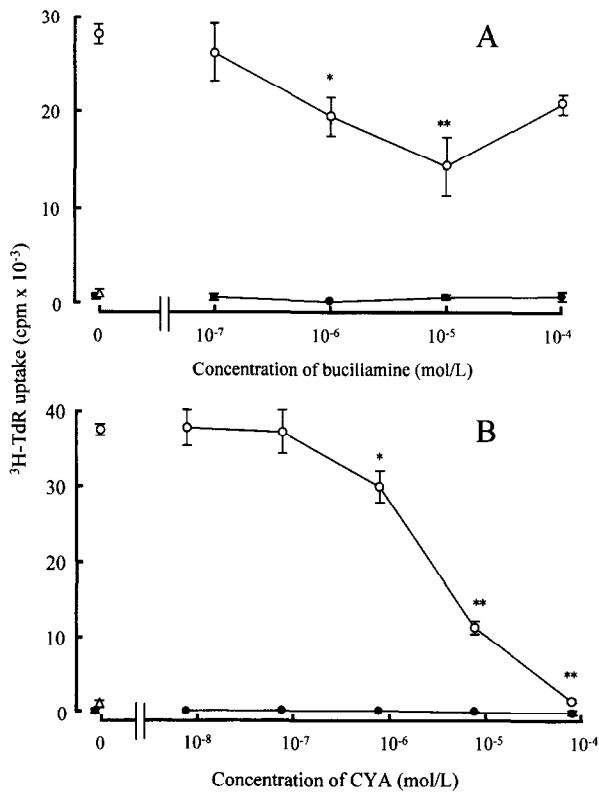
**Table 1.** Effects of Bucillamine on Antigen-Presenting Activity of Macrophages

Culture Composition		Treatment for Macrophages			$^3$ H-TdR Uptake (cpm $\times 10^{-3}$ )
		Pretreatment	Treatment (120 min)		
T Cells	Macrophages	Bucillamine (min)	S-antigen	Bucillamine	
+	+	0	+	-	85.3 $\pm$ 1.6
+	+	0	+	+	84.7 $\pm$ 1.5
+	+	5	+	+	81.2 $\pm$ 2.7
+	+	30	+	+	73.5 $\pm$ 4.5 <sup>a</sup>
+	+	60	+	+	54.0 $\pm$ 5.3 <sup>b</sup>
+	+	120	+	+	34.7 $\pm$ 1.5 <sup>b</sup>
+	+	0	-	-	10.0 $\pm$ 2.3 <sup>b</sup>
+	-	0	-	-	2.9 $\pm$ 1.0 <sup>b</sup>
-	+	0	-	-	1.0 $\pm$ 0.3 <sup>b</sup>

Each value represents mean  $\pm$  SE of four samples.

<sup>a</sup> $P < 0.05$ .

<sup>b</sup> $P < 0.01$  versus first line in data (Dunnett's multiple comparison test).



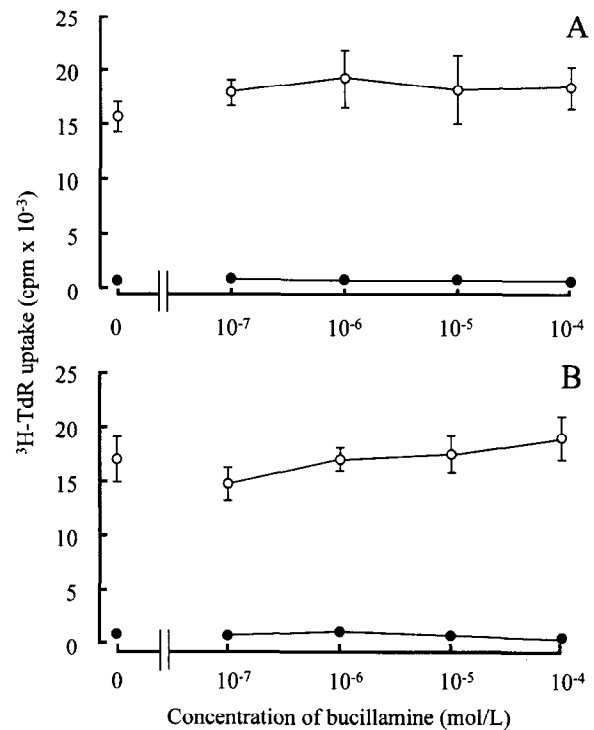
**Figure 2.** Effects of bucillamine (A) and CYA (B) on antigen-presenting activity of macrophages. Macrophages were cultured with several concentrations of drugs for 4 hours and pulsed by S-antigen for last 2 hours. Treated macrophages and S-antigen responsive T cells were co-cultured for 4 days and primed with <sup>3</sup>H-thymidine (TdR) for last 16 hours. Antigen-presenting activity was expressed as incorporated radioactivity. ○: co-culture with macrophages and T cells; ●: single culture with macrophages, △: single culture with T cells. Each value represents mean ± SE of four samples. \**P* < 0.05, \*\**P* < 0.01 versus untreated culture (Dunnett's multiple comparison test).

in a dose-dependent manner ( $10^{-7}$ – $10^{-5}$  mol/L) and the maximum inhibition was achieved at  $10^{-5}$  mol/L of bucillamine. The <sup>3</sup>H-TdR uptake by the macrophage fraction was minimal at any concentration of bucillamine (Figure 2A). However, pre- and post-treatment with bucillamine on the S-antigen-pulsed macrophage fraction did not affect the <sup>3</sup>H-TdR uptake by the culture (Figure 3).

<sup>3</sup>H-TdR uptake by the culture was also inhibited by treatment of the macrophage fraction with CYA at concentrations from  $8.3 \times 10^{-7}$  mol/L (1 μg/mL) to  $8.3 \times 10^{-5}$  mol/L (100 μg/mL; Figure 2B).

#### Drug effects on proliferative response of T cells.

The <sup>3</sup>H-TdR uptake by the culture consisting of the S-antigen sensitized T-cell fraction and macrophage fraction was not suppressed by pretreatment of the

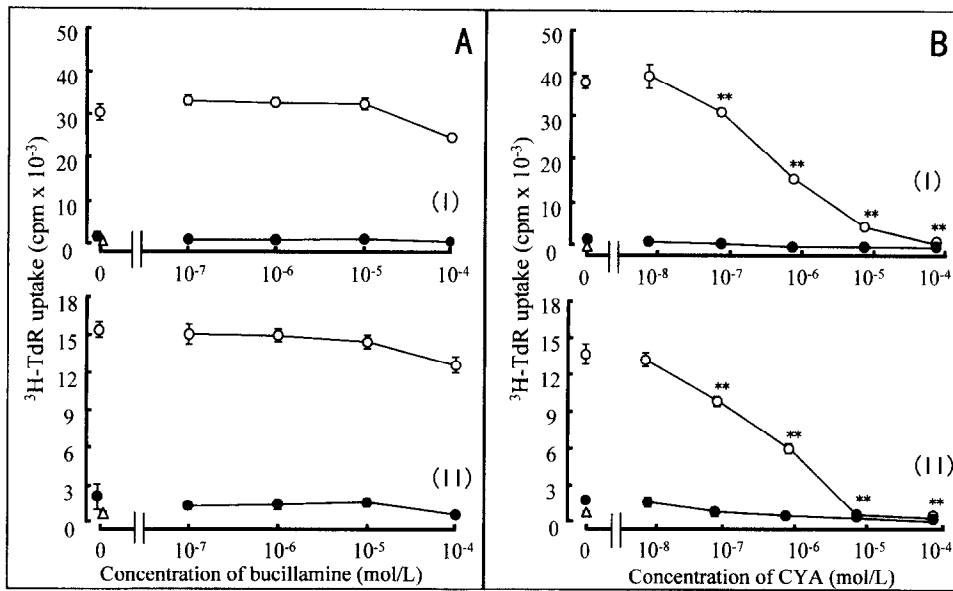


**Figure 3.** Effects of pre- (A) or post-treatment (B) with bucillamine on antigen-presenting activity of macrophages. Macrophages pulsed by S-antigen for 2 hours were pre- or post-treated with several concentrations of bucillamine for 4 hours. Treated macrophages and S-antigen responsive T-cells were co-cultured for 4 days and primed with <sup>3</sup>H-TdR for the last 16 hours. Antigen-presenting activity was expressed as incorporated radioactivity. ○: co-culture with macrophages and T cells, ●: single culture with macrophages. Each value represents mean ± SE of four samples.

T-cell fraction with bucillamine (Figure 4A). The in vitro treatment of the T-cell fraction with CYA, however, greatly suppressed the <sup>3</sup>H-TdR uptake in a dose-dependent manner at concentrations higher than  $8.3 \times 10^{-8}$  mol/L (0.1 μg/mL; Figure 4B).

**Effect of bucillamine on pinocytotic activity of macrophages.** The mean fluorescence values of the macrophage fraction were not affected by treatment of the fraction with bucillamine (Figure 5). However, the fluorescence value in the control group, incubated at a low temperature, was markedly decreased.

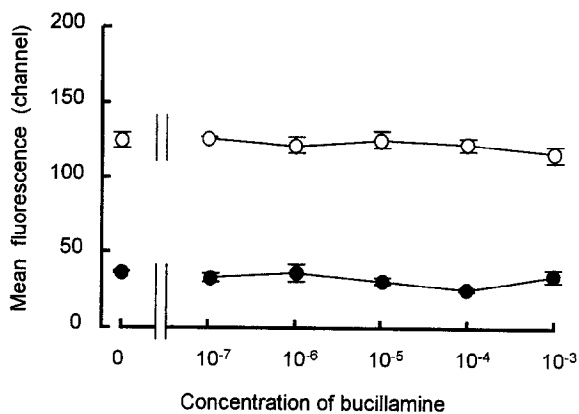
**Effect of bucillamine on antigen-presenting activity of RPE cells to T cells.** The <sup>3</sup>H-TdR uptake by the S-antigen-sensitized T-cell fraction co-cultured with the S-antigen-pulsed RPE cells was significantly increased in comparison with that of the T-cell fraction or RPE cells alone (Table 2). The in vitro treatment with bucillamine on RPE cells reduced the <sup>3</sup>H-TdR uptake in a dose-dependent manner at concentrations between  $10^{-7}$  mol/L and  $10^{-3}$  mol/L (Table 2).



**Figure 4.** Drug effects on antigen-specific proliferation of T cells. S-antigen responsive T cells were cultured with bucillamine (A) or CYA (B) for 4 hours (I) or 24 hours (II). Treated T cells and S-antigen pulsed macrophages were co-cultured for 4 days and primed with <sup>3</sup>H-TdR for the last 16 hours. T-cell proliferation was expressed as incorporated radioactivity. O: co-culture with T cells and macrophages, ●: single culture with T cells, Δ: single culture with macrophages. Each value represents mean ± SE of four samples. \*\**P* < 0.01 versus untreated culture (Dunnett's multiple comparison test).

### In Vivo Therapy

Systemic administration of bucillamine or CYA for 14 days post-immunization with S-antigen completely suppressed the development of EAU in drug-treated rats, while all control untreated rats developed EAU (data not shown).



**Figure 5.** Effects of bucillamine on pinocytotic activity of macrophages. Macrophages were cultured with several concentrations of bucillamine for 4 hours, and pulsed with FITC conjugated S-antigen for 30 minutes at 37°C (O). As control, bucillamine-treated macrophages were pulsed with FITC-conjugated-S-antigen at 10°C (●). Pinocytotic activity of macrophages was expressed as mean fluorescence level of macrophages. Each value represents the mean ± SE of four samples.

### Effects of bucillamine on antigen-presenting activity of macrophages.

The <sup>3</sup>H-TdR uptake by the T-cell fraction from S-antigen-immunized and untreated rats (control rats) was remarkably high when co-cultured with S-antigen-pulsed macrophages from the control rats (Table 3). The <sup>3</sup>H-TdR uptake by the culture consisting of the T-cell fraction from control rats and the macrophage fraction from bucillamine-treated rats was significantly suppressed. The suppression was dose-dependent and the difference between the untreated group and the 200 mg/kg bucillamine group was statistically significant (*P* < 0.05). In contrast, treating rats with 10 mg/kg of CYA did not affect the level of <sup>3</sup>H-TdR uptake.

### Effects of bucillamine on proliferation of T cells.

In contrast to the previous experiment, in vivo therapy with bucillamine did not suppress the <sup>3</sup>H-TdR uptake by the culture of macrophage fraction from the control rats and the T-cell fraction from the bucillamine-treated rats (Table 4). However, treating rats with CYA greatly suppressed the <sup>3</sup>H-TdR uptake (Table 4).

## Discussion

The data recorded here demonstrated the unique immunopharmacological activities of bucillamine which differ from those of CYA. In vitro and in vivo experiments showed that bucillamine was capable of

**Table 2.** Effects of Bucillamine on Antigen-Presenting Activity of RPE Cells to T Cells

Culture Composition		Treatment to RPE		<sup>3</sup> H-TdR Uptake (cpm × 10 <sup>-3</sup> )
T Cells	RPE Cells	Bucillamine (mol/L)	S-antigen	
+	+	0	+	3.36 ± 0.47
+	+	10 <sup>-7</sup>	+	2.28 ± 0.25 <sup>a</sup>
+	+	10 <sup>-6</sup>	+	1.86 ± 0.21 <sup>b</sup>
+	+	10 <sup>-5</sup>	+	1.88 ± 0.07 <sup>b</sup>
+	+	10 <sup>-4</sup>	+	1.81 ± 0.32 <sup>b</sup>
+	+	10 <sup>-3</sup>	+	1.59 ± 0.30 <sup>b</sup>
-	+	0	+	0.33 ± 0.07 <sup>b</sup>
+	-	0	-	0.35 ± 0.01 <sup>b</sup>
+	+	0	-	0.86 ± 0.16 <sup>b</sup>

Each value represents mean ± SE of three samples.

<sup>a</sup>P < 0.05.

<sup>b</sup>P < 0.01 versus first line in data (Dunnett's multiple comparison test).

suppressing the antigen-presenting activity of macrophages obtained from spleen, but not the antigen-specific proliferation of T cells. On the other hand, in vitro treatment of T cells with CYA suppressed both the antigen-presenting activity of macrophages and the antigen-specific proliferation of T cells. The concentration of CYA needed to suppress the antigen-presenting activity was tenfold higher than that needed to suppress antigen-specific T-cell proliferation, indicating a much higher capacity of CYA to suppress T cells than macrophages. This hypothesis was further supported by in vivo experiments, where therapy of S-antigen-immunized rats with CYA suppressed antigen-specific proliferation of T cells, but not the antigen-presenting activity of macrophages.

It is not yet determined what type of intraocular resident cells have antigen-presenting activity. Recent studies suggest that RPE cells may play a role as APCs, as demonstrated mainly by Ia-antigen expression in RPE cells by immunohistopathological exam-

inations.<sup>15-18</sup> We investigated the antigen-presenting activity of RPE cells by co-culturing RPE cells and S-antigen-sensitized T cells with S-antigen, and the effects of bucillamine on the activity. The results in the present study demonstrated that rat RPE cells do have the capacity to present S-antigen to T cells, and that the antigen-presenting activity of RPE cells was suppressed by bucillamine in a dose-dependent manner. Thus bucillamine is capable of suppressing antigen-presenting activity of two different APCs.

No studies on the effects of bucillamine on antigen-presenting activity have been reported to date, and the mechanisms by which bucillamine exhibits this activity has not yet been determined. We investigated the immunopharmacological mechanisms of the drug. Bucillamine suppressed the antigen-presenting activity of macrophages only when it was co-cultured with S-antigen and macrophages for a certain period of time. Macrophages which were pretreated with bucillamine and stimulated with S-antigen after wash-

**Table 3.** Effects of In Vivo Therapy With Bucillamine on Antigen-Presenting Activity of Macrophages

Drug Treatment of Donor Rats for Macrophages	Culture Composition		<sup>3</sup> H-TdR Uptake (cpm × 10 <sup>-3</sup> )
	Macrophages	T Cells	
None	+	+	48.22 ± 6.39
Bucillamine 20 mg/kg	+	+	31.03 ± 4.83
Bucillamine 200 mg/kg	+	+	25.01 ± 4.11 <sup>a</sup>
CYA 10 mg/kg	+	+	47.54 ± 5.92
None	+	-	1.55 ± 0.31 <sup>b</sup>
None	-	+	0.27 ± 0.03 <sup>b</sup>

Macrophages were obtained from drug-treated or untreated rats. T cells were obtained from untreated rats. Each value represents mean ± SE of four animals.

<sup>a</sup>P < 0.05.

<sup>b</sup>P < 0.01 versus first line in data (Dunnett's multiple comparison test).

**Table 4.** Effects of In Vivo Therapy With Bucillamine on Antigen-Specific T Cell Proliferation

Treatment of Donor Rats for T Cells	Culture Composition		<sup>3</sup> H-TdR Uptake (cpm × 10 <sup>-3</sup> )
	T Cells	Macrophages	
None	+	+	47.33 ± 6.07
Bucillamine 20 mg/kg	+	+	54.75 ± 3.43
Bucillamine 200 mg/kg	+	+	55.85 ± 5.20
CYA 10 mg/kg	+	+	8.43 ± 1.00 <sup>a</sup>
None	+	-	0.46 ± 0.04 <sup>a</sup>
None	-	+	1.02 ± 0.27 <sup>a</sup>

T cells were obtained from drug-treated or untreated rats. Macrophages were obtained from untreated rats. Each value represents mean ± SE of four animals.

<sup>a</sup>*P* < 0.01 versus first line in data (Dunnett's multiple comparison test).

ing out bucillamine in the medium retained the antigen-presenting activity when cultured with T cells. This indicates that bucillamine has no effects on macrophages per se. Macrophages which were pretreated with S-antigen and then co-cultured with bucillamine after washing out S-antigen in the medium also retained the antigen-presenting activity when cultured with T cells. This indicates that bucillamine does not affect the macrophages which have already reacted with antigen and expressed the antigen-related peptides on the major histocompatibility complex (MHC) antigen at the cellular surface. These data thus suggest that bucillamine has an effect between the process where S-antigen is taken into macrophages and the process where S-antigen-related peptides are expressed on the MHC antigen at the cellular surface. Because S-antigen is a water-soluble protein, macrophages are considered to take S-antigen into the cells by pinocytosis. Whether bucillamine might affect the pinocytotic activity of macrophages was tested by an in vitro experiment using FITC-conjugated S-antigen. It was shown that bucillamine did not suppress the pinocytotic activity of macrophages. Much further investigation is needed to determine the mechanisms by which bucillamine causes the unique immunopharmacological activity on APCs.

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