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Roxithromycin promotes lymphocyte apoptosis in *Dermatophagoides*-sensitive asthma patients

Norifumi Ogawa*, Yoko Sugawara, Yukiko Fujiwara, Takeshi Noma

Department of Pediatrics, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan

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Abstract

A new macrolide, roxithromycin, may be an effective treatment for asthma. Lymphocyte apoptosis is impaired in patients with asthma, while spontaneous apoptosis increases during remission, and such changes may be involved in the onset and remission of mite-sensitive asthma. Lymphocyte apoptosis was evaluated by incubating cells from patients with asthma in the presence of roxithromycin. Low concentrations of roxithromycin (1–500 ng/ml) augmented the early, but not late, phase of apoptosis in *Dermatophagoides farinae*-stimulated peripheral blood mononuclear (PBM) cells, while high concentrations of roxithromycin (1 μg/ml; 6 μg/ml is the maximum serum level) augmented both the early and late phases of apoptosis. In both unstimulated and phytohemagglutinin-stimulated cells, roxithromycin did not significantly affect the induction of apoptosis. In cells from normal subjects, roxithromycin did not affect the induction of apoptosis. Other antibiotics, including cefazolin and ampicillin, did not cause significant induction of apoptosis. Fas ligand, but not Fas receptor, expression on *D. farinae*-stimulated cells was up-regulated after stimulation with 1 μg/ml roxithromycin, while Bcl-2 expression on both unstimulated and *D. farinae*-stimulated cells showed a decrease after the same treatment. Roxithromycin can induce apoptosis of *D. farinae*-activated lymphocytes in patients with *D. farinae*-sensitive asthma. Induction of the Fas/Fas ligand system and reduced Bcl-2 expression were involved in the promotion of apoptosis by roxithromycin treatment.

Keywords: Roxithromycin; Apoptosis; Asthma; Fas ligand; Bcl-2

1. Introduction

Roxithromycin (Fig. 1) has a molecular weight of 837.06, and may be an effective treatment for asthma (Konno et al., 1994; Shimizu et al., 1994). This new macrolide increases the phagocytotic and bactericidal activity of neutrophils (Yokota and Bhattacharyya, 1988; Noma et al., 1998). Clinical studies have shown its efficacy for chronic diseases, including diffuse panbronchiolitis (Kusano et al., 1995; Sakito et al., 1996; Nakamura et al., 1999) and chronic sinusitis (Kimura et al., 1997). Therapeutic value for allergic diseases, such as atopic dermatitis (Wakita et al., 1996) and asthma (Konno et al., 1994; Shimizu et al., 1994), has also been suggested.

In vitro studies have revealed that roxithromycin modifies the functions of neutrophils (Yokota and Bhattacharyya, 1988; Konno et al., 1992; Abdelghaffar et al., 1996; Noma et al., 1998; Nakamura et al., 1999) and keratinocytes (Wakita et al., 1996).

Roxithromycin also alters lymphocyte proliferation induced by mitogens and purified protein derivative (PPD) (Konno et al., 1992), as well as proliferation and cytokine secretion induced by mitogens (Konno et al., 1994; Yoshimura et al., 1995). The unbalanced cytokine profile of patients with mite antigen-induced asthma may be corrected by roxithromycin, which may reproduce the changes of the immune system seen in patients in remission who are tolerant of *Dermatophagoides farinae* antigen (Noma et al., 2002).

Apoptosis, or programmed cell death, is a genetic program that leads to the death and efficient disposal of cells (Wyllie et al., 1980; Korsmeyer, 1995; Cohen, 1996; Gupta, 1996), and is essential for normal development and cellular homeostasis (Nagata, 1996; Osborne, 1996). In the immune system, apoptosis appears to play an important role in the thymic selection of T cells and in the development of peripheral tolerance (Cohen, 1996; Osborne, 1996). In contrast, dysregulation of apoptosis may lead to the development of autoimmune diseases (Lopez-Hoyos et al., 1998; Ravirajan et al., 1999; Zhou et al.,1999) and allergic diseases (Noma et al., 2002). The present study examined

* Corresponding author. Tel.: +81-42-778-8111; fax: +81-42-778-8441.
E-mail address: nomatake@med.kitasato-u.ac.jp (N. Ogawa).

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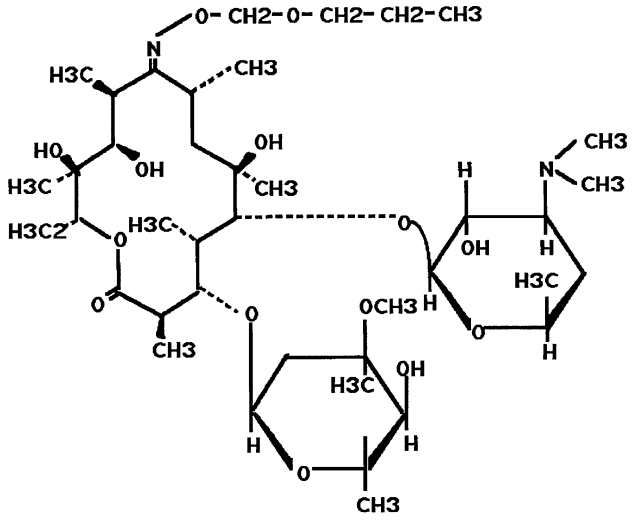

Fig. 1. Structure of roxithromycin.

the anti-allergic properties of roxithromycin, focusing on its effect on *D. farinae*-induced apoptosis in patients with asthma.

2. Materials and methods

2.1. Subjects

PBM cells were obtained from seven patients with active asthma and four non-allergic healthy volunteers who served as a control group. The diagnostic criteria for asthma used in this study were those established by the American Thoracic Society. Patients with active asthma had recurrent exacerbations and a positive immediate skin reaction, as defined by an immediate wheal response in a skin prick test (mean diameter $\geq$ 10 mm) for house dust mite and *D. farinae* antigens (mite 1, Torii & Co., Tokyo, Japan). Active asthma was defined as 10 or more mild-to-moderate attacks per year despite treatment with theophylline (15–20 mg/kg/day), a β2-adrenoceptor agonist, or both. The total serum immunoglobulin E (IgE) concentration and the IgE score for mite antigen (Miyamoto et al., 1974) determined by radioallergosorbent testing were 1166 ± 599 IU/ml and 3– 6, respectively, in the patients with active asthma. At the time of the study, patients were not suffering from an acute exacerbation and were not taking oral corticosteroids or anti-allergy agents, such as ketotifen (Noma et al., 1990). Patients in remission had been disease-free for more than 2 years without the need for medication. The total serum IgE concentration and the IgE score for mite antigen were 1185 ± 383 IU/ml and 4–6, respectively, in these patients. Patients were prospectively followed up until their disease status (active or remission) could be determined. In both groups, the flow expiratory volume 1 (FEV1) was within normal limits between acute asthma attacks and during the 4- to 6-week period before examination. During the 2-year period before remission, asthma had been mild to moderate in the patients with remission.

The overall severity of asthma and the severity of acute exacerbations were estimated using the Guidelines for the Diagnosis and Management of Asthma (the National Asthma Education Program Expert Panel Report) released by the National Institutes of Health, USA. A mild exacerbation was defined as the presence of normal alertness with absent or mild dyspnea (patient was able to speak in complete sentences), no or mild intercostal retraction, normal skin color, wheezing limited to end-expiration and a peak expiratory flow rate (PEFR) or FEV1 that was 70–90% of the predicted value. The clinical characteristics of the two patient groups are summarized in Table 1.

Informed consent was obtained from each patient or from the patient's parents; moreover, this study was approved by our Institutional Review Board.

2.2. Assay of apoptosis

Apoptotic PBM cells were detected by annexin V staining (Reutelingsperger and Van Heerde, 1997; Van Engeland et al., 1998; Bedner et al., 1999) using a Mebcyto Apoptosis Kit (MBL, Nagoya, Japan) according to the manufacturer's instructions. Briefly, cells were washed in phosphate-buffered saline (PBS; pH 7.2) and assessed for viability by Trypan blue dye exclusion, after which they were plated at 2×10^5 viable cells/well (200 μl). Then the cells were incubated for 72 h in culture medium alone (RPMI 1640 with 10% fetal calf serum) or in medium containing 1 μg/ml *D. farinae* antigen (Torii & Co.) or 0.5% phytohemagglutinin (Sigma, St. Louis, MO). After harvesting, the cells were washed with PBS and resuspended in 85 μl of binding buffer, followed by the addition of 10 μl of annexin V-Fluorescein Isothiocyanate (FITC) (1 μg/ml) and 5 μl of propidium iodide (PI) (10 μg/ml). Then the cells were resuspended and incubated at room temperature for 15 min in the dark,

Table 1
Characteristics of subjects used in the study

	Controls	Active	Remission
No. of patients	4	7	6
Age (years): median, range	7, 5–9	10, 5–15	15, 11–18
Sex (M:F)	2:2	5:2	4:2
Disease severity	(non-allergy)	mild	disease-free
Documented attacks[a], more than 10	–	(per year)	mild or moderate
Medication	–	+	(intermittent or persistent)
Serum IgE (U/ml)	92 ± 43[b]	1166 ± 599	1185 ± 383
Df-IgE RAST score	0 ± 0	4.5 ± 0.3	5.0 ± 0.7
Eosinophil count (/ml)	NT	546 ± 88	451 ± 180
Skin prick test (Df)	–	+	+

[a] Per year over the previous 2 years.
[b] Mean value ± standard error.

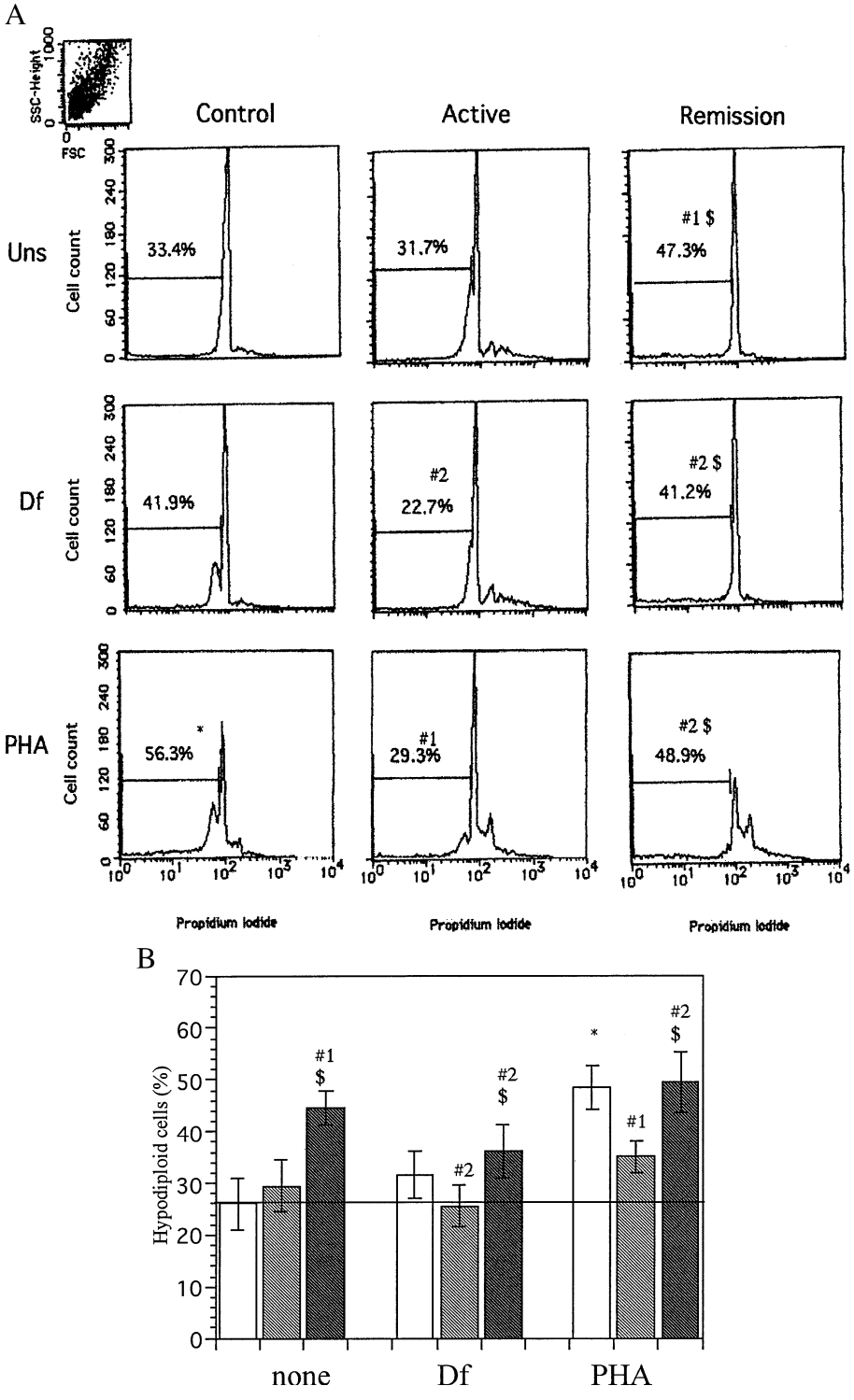

Fig. 2. Induction of PBMcell apoptosis in asthma patients. (A) Representative FACS analysis of hypodiploid cells stained with propidium iodide. The percentage of cultured cells undergoing apoptosis was quantified by flow cytometry to identify fragmented nuclei. Uns, *Df*, PHA: unstimulated, *Dermatophagoides farinae* stimulation, phytohemagglutinin stimulation. Control: a normal subject; Active: an active asthma patient; Remission: a patient in remission. *: $P<0.05$ (compared to unstimulated), #1: $P<0.05$ (compared to controls), #2: $P<0.05$ (compared to controls, by stimulation index against unstimulated), $: $P<0.05$ (compared to patients). (B) Percentage of hypodiploid cells stained with propidium iodide. Data from seven independent experiments were pooled. □: normal subjects ($n=4$); ▨: active subjects ($n=7$); ■: patients in remission ($n=6$). *: $P<0.05$ (compared to unstimulated). #1: $P<0.05$ (compared to controls), #2: $P<0.05$ (compared to controls, by stimulation index against unstimulated), $: $P<0.05$ (compared to patients). (C) Induction of PBM cells apoptosis detected by annexin V staining. To verify the induction of apoptosis in cells from patients in remission, cells were stained with annexin V-FITC and propidium iodide. Next, stained cells were analyzed by a FACScan. Representative data from four independent experiments are shown. Uns, *Df*, PHA: unstimulated, *D. farinae*- or phytohemagglutinin-stimulated PBM cells were cultured for 72 h. *: $P<0.05$ (compared to unstimulated).

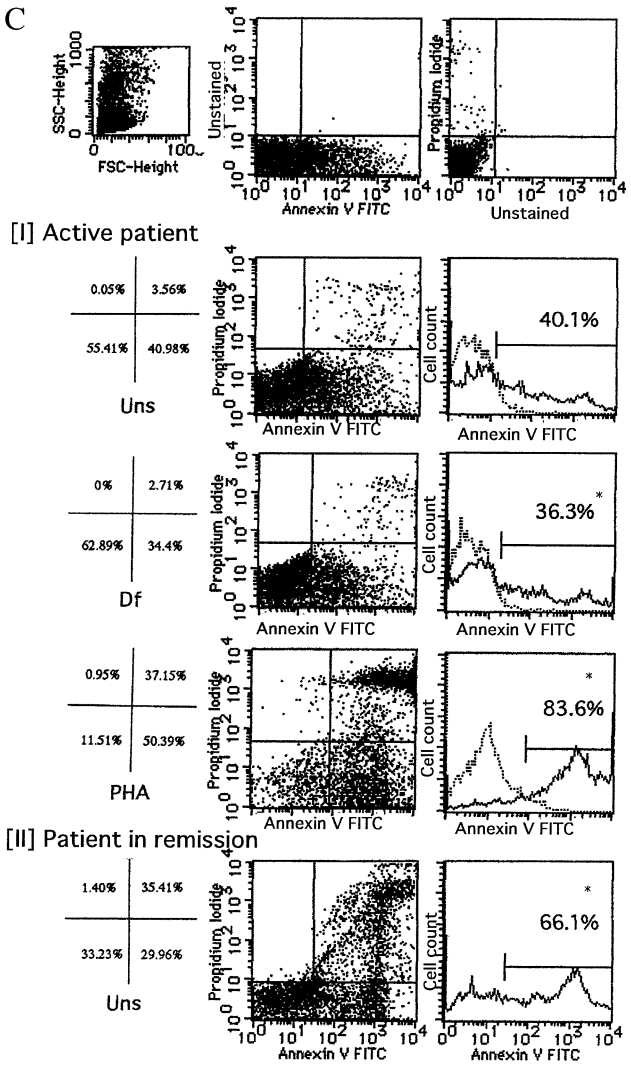

Fig. 2 (*continued*).

after which the stained cells were analyzed by flow cytometry within 1 h.

The percentage of apoptotic cells was also quantified with a modification of the flow cytometric method used to detect fragmented nuclei with propidium iodide staining (Nicoletti et al., 1991; Oyaizu et al., 1993; Laurence et al., 1996). Briefly, the cells were harvested, washed, fixed in 70% cold ethanol, and incubated for 16 h at −20 °C with propidium iodide (50 μg/ml; Sigma) in the presence of RNase A (300 U/ml, Sigma).

2.3. Fas receptor expression

Mononuclear cells (2×10^5) were plated for 72 h in culture medium alone or medium with 1 μg/ml *D. farinae* or 0.5% phytohemagglutinin. Then the cells were washed twice with PBS and incubated for 45 min on ice with an FITC-labeled anti-Fas (CD95) monoclonal antibody (Medical and Biological Laboratories, Nagoya, Japan) or an isotypic control antibody. The cells were washed again and Fas receptor expression was determined by flow cytometry.

2.4. Fas-ligand expression

Mononuclear cells (2×10^5) were cultured for 72 h (37 °C, 10% CO_2) in 1 ml of RPMI 1640 medium containing 40 μg/ml gentamycin and 10% fetal calf serum (Gibco BRL, Gaithersburg, MD) with stimulation by 1 μg/ml *D. farinae* or 0.5% phytohemagglutinin (Sigma) plus 0.1 units of recombinant interleukin-2 (Takeda). *D. farinae*-stimulated cells were then cultured for an additional 16 h in the presence of a metalloprotease inhibitor, Tissue inhibitor of metalloproteinase (TIMP) 1, 2 (Calbiochem, San Diego, CA) (Boone et al., 1990; Liotta and Stetler-Stevenson, 1990), while cells stimulated by phytohemagglutinin plus interleukin-2 were cultured with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) and 500 ng/ml ionomycin (Sigma) in the presence of TIMP 1, 2 (Calbiochem). After incubation, the cells were incubated for 45 min on ice with a biotinylated anti-human Fas ligand (CD95L) monoclonal antibody (Pharmingen) or with mouse IgG2a (as an isotype control). Then the cells were washed with PBS containing 1% fetal calf serum and 0.1% sodium azide, and stained for 30 min on ice with a streptavidin–fluorescein conjugate (Pharmingen). After further washing with PBS, 10,000 cells were analyzed for Fas-ligand expression by flow cytometry.

2.5. Bcl-2 expression

Bcl-2, an intracellular antigen that localizes predominantly to the outer mitochondrial membrane (Reed, 1994), was quantified in fixed PBM cells. Mononuclear cells (2×10^5) were cultured for 72 h in culture medium alone (RPMI 1640 with 10% fetal calf serum) or medium with 1 μg/ml *D. farinae* antigen (Torii & Co.) and were subsequently fixed by incubation with 1% paraformaldehyde for 15 min at room temperature and then with 70% methanol for 45 min on ice. Then the cells were washed twice with PBS and incubated for 45 min on ice with an FITC-conjugated mouse anti-human Bcl-2 oncoprotein monoclonal antibody (Dako Japan, Kyoto, Japan) or with FITC-conjugated mouse IgG1 as an isotype control. After further washing with PBS, the cells were analyzed by flow cytometry, and the results were reported as the mean ± standard error (S.E.) of the fluorescence channel intensity in Bcl-2^+ cells.

2.6. Treatment with roxithromycin

Serial dilutions of roxithromycin dissolved in RPMI 1640 medium (adjusted to pH 7.4) were added to PBM cells (1×10^6/ml), after which the cells were stimulated with antigen or mitogen. Responses to stimulation were assessed, including the induction of apoptosis and the expression of Fas receptors, Fas ligand, and Bcl-2.

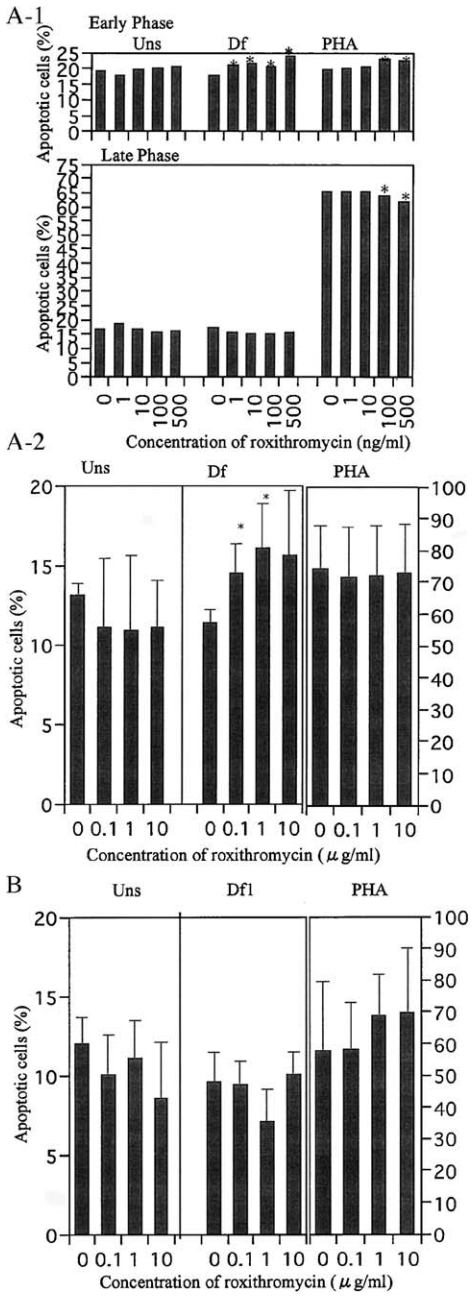

Fig. 3. Effect of roxithromycin on apoptosis. Serial dilutions of roxithromycin dissolved in RPMI 1640 medium were added to PBM cells, after which the cells were stimulated with *D. farinae* antigen or phytohemagglutinin. The induction of apoptosis was assessed by annexin V and propidium iodide staining. (A) Patients. Data show low concentrations of roxithromycin (1–500 ng/ml) in the early phase (annexin-positive, propidium iodide-negative cells), and in the late phase (annexin-positive, propidium iodide-positive cells), of apoptosis in patient PBM cells (A-1), and show effect of roxithromycin (0.1, 1, 10 μg/ml) in the early plus late phase of apoptosis in patient PBM cells (A-2). A-1—Representative data of three independent experiments is shown. $*P<0.05$ (as stimulation index, compared to 0 ng/ml roxithromycin, $n=3$). A-2—Data of seven independent experiments pooled. $*P<0.05$ (compared to 0 μg/ml roxithromycin). (B) Normal subjects. Data show effect of roxithromycin (0.1, 1, 10 μg/ml) in the early plus late phase of apoptosis in normal subjects. Data of four independent experiments pooled. $P<0.05$ (compared to 0 μg/ml roxithromycin).

2.7. Statistical analysis

Results are expressed as the means ± S.E. Data were analyzed by analysis of variance (ANOVA) with Bonferonni's correction, or the Wilcoxon test (Fig. 5). Differences of $P<0.05$ were considered statistically significant.

3. Results

3.1. Induction of apoptosis in PBM cells from asthma patients

In normal individuals, hypodiploid cells accounted for 25.9 ± 4.9% of unstimulated PBM cells, which was similar to the percentage in the patients with active asthma (29.5 ± 5.0%; Fig. 2A and B). After *D. farinae* stimulation, the percentage of hypodiploid cells was decreased significantly in patients with active asthma (0.95 ± 0.06) when compared to that in normal individuals (1.31 ± 0.16; Fig. 2B). Similar findings were observed after phytohemagglutinin

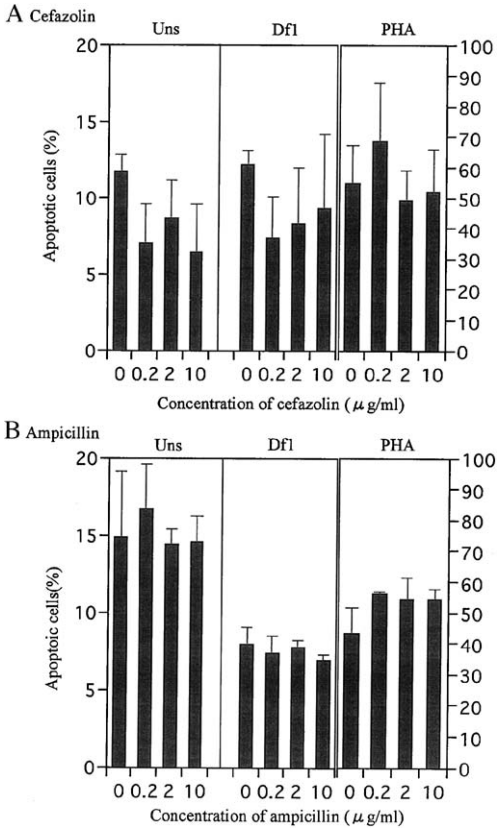

Fig. 4. Lack of significant induction of apoptosis by cefazolin and ampicillin. Serial dilutions of cefazolin (A) and ampicillin (B), were added to patient PBMcells, after which the cells were stimulated with *D. farinae* antigen or phytohemagglutinin. The early plus late phase of apoptosis was assessed by annexin V and propidium iodide staining. Data of four independent experiments pooled.

stimulation, and the percentage of hypodiploid cells (35.1 ± 3.2%) was significantly decreased in the patients with active asthma when compared to that (48.5 ± 4.3%) in normal individuals (Fig. 2A and B).

To verify the induction of apoptosis, cells were stained with FITC-conjugated annexin V. The results were similar to those obtained with propidium iodide staining (Fig. 2C).

3.2. Effect of roxithromycin on apoptosis of PBM cells from asthma patients

Low concentrations of roxithromycin (1–500 ng/ml) augmented the early phase (annexin-positive, propidium iodide-negative cells), but not the late phase (annexin-positive, propidium iodide positive cells), of apoptosis in *D. farinae*-stimulated PBM cells (Fig. 3A-1), while a high

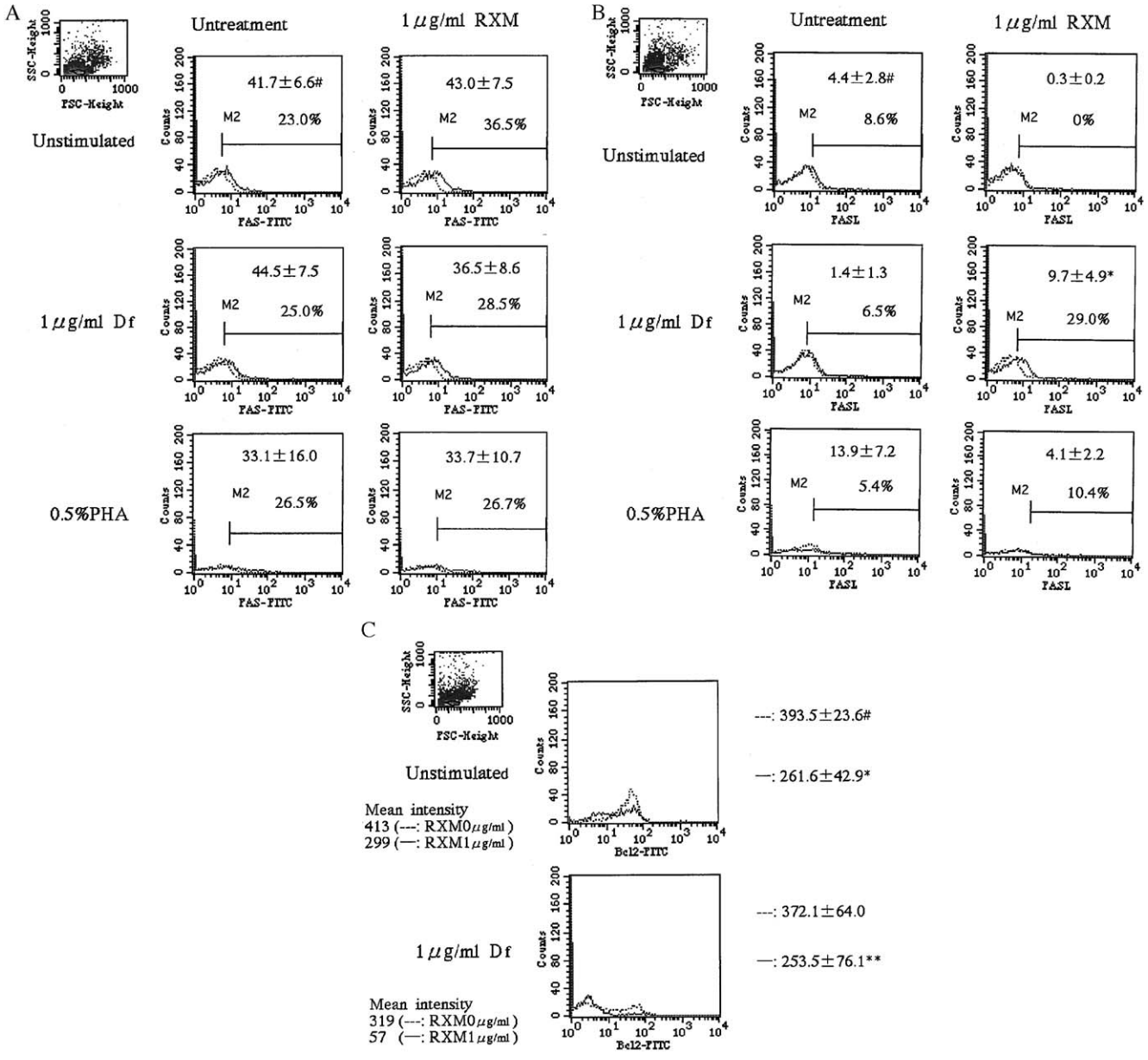

Fig. 5. Expression of Fas receptor, Fas ligand, and Bcl-2 by lymphocytes from asthma patients. (A) Representative FACS analysis of Fas receptor expression by PBM cells. Uns, *Df*, PHA: unstimulated, *D. farinae* stimulation, phytohemagglutinin stimulation. Solid lines: anti-Fas; dotted lines: isotypic control antibody. #: Data show the means ± S.E. of five independent experiments. (B) Fas ligand expression by PBM cells. Uns, *Df*, PHA: unstimulated, *D. farinae* stimulation, phytohemagglutinin stimulation. Solid lines: anti-Fas ligand, Dotted lines: isotypic control antibody. #: Data show the means ± S.E. of five independent experiments. *: $P = 0.047$ (compared to untreated). (C) Bcl-2 expression by PBM cells. Intracellular expression of Bcl-2 was determined using CellQuest software. Results are represented as the mean ± standard error of the fluorescence channel intensity in Bcl-2^+ cells. #: Data show the means ± S.E. of five independent experiments. *: $P = 0.01$ (compared to untreated). **: $P = 0.049$ (compared to untreated).

concentration of roxithromycin (1 μg/ml; 6 μg/ml is the maximum serum level) augmented both the early and late phases of apoptosis (Fig. 3A-2). In unstimulated cells, low concentrations of roxithromycin (1–500 ng/ml) did not affect the induction of apoptosis, and the apoptosis induced by a high concentration of roxithromycin was variable (Fig. 3A-2). In phytohemagglutinin-stimulated cells, there was a significantly augmented effect of 100 and 500 nM of roxithromycin in the early phase (Fig. 3A-1), and a reduced effect in the late phase in co-stimulated cells (see Fig. 3A-1), but in Fig. 3A-2, there is no significant effect of 100 nM of roxithromycin plus phytohemagglutinin in the early plus late phase apoptosis. The absence of effect of 100 nM of roxithromycin likely reflects cancellation of the effect of roxithromycin in the early and late phase apoptosis. These data showed that roxithromycin did not significantly influence the induction of apoptosis in phytohemagglutinin-stimulated cells.

In normal subjects, roxithromycin did not affect the induction of apoptosis (Fig. 3B). Other antibiotics, including cefazolin and ampicillin, did not cause significant induction of apoptosis (Fig. 4). These results indicated that roxithromycin could induce apoptosis of *D. farinae*-activated lymphocytes in patients with *D. farinae*-sensitive asthma.

3.3. Expression of Fas receptor, Fas ligand, and Bcl-2

Fas receptor expression by unstimulated cells was enhanced after culture with 1 μg/ml of roxithromycin for 3 days. However, the differences between the means of the five independent experiments with five patients were not significant. After stimulation with 1 μg/ml *D. farinae* antigen or 0.5% phytohemagglutinin, Fas receptor expression was also unchanged (Fig. 5A).

Fas ligand expression by unstimulated or phytohemagglutinin-stimulated cells was not affected by the addition of roxithromycin, whereas stimulation with 1 μg/ml *D. farinae* antigen significantly increased the expression of Fas ligand by cells from patients (Fig. 5B). These results indicated that the expression of Fas ligand by *D. farinae*-stimulated cells was up-regulated in patients with active asthma.

Expression of Bcl-2 in both unstimulated and *D. farinae*-stimulated cells was decreased by the same treatment (Fig. 5C).

4. Discussion

Roxithromycin induced the early phase of apoptosis in activated cells, after which the cells proceeded to the late phase of apoptosis, since low concentrations of roxithromycin (1–500 ng/ml) augmented the early, but not the late, phase of apoptosis in *D. farinae*-stimulated PBM cells. However, a higher concentration of roxithromycin (1 μg/ml), the maximum clinical level of which is approximately 6 μg/ml, augmented both the early and late phases of apoptosis in *D. farinae*-stimulated cells. In unstimulated cells, roxithromycin did not significantly affect apoptosis. In contrast, roxithromycin did not induce apoptosis in normal subjects. The combined results suggest that roxithromycin can induce apoptosis when lymphocytes are activated by a relevant antigen in patients with asthma.

Allergen-activated lymphocytes accumulate in the bronchi due to an increased survival time and thus maintain inflammation in patients with mite-sensitive asthma. Apoptosis induced by *D. farinae* allergen was decreased in lymphocytes from patients with mite-sensitive asthma, and spontaneous apoptosis as well as *D. farinae*-stimulated apoptosis was markedly increased among patients with asthma in remission (Noma et al., 2002). Clearance of $CD8^+$ T cells by increased apoptosis is likely to be involved in outgrowing asthma (Noma et al., 2002).

D. farinae stimulation induced apoptosis of activated cells in normal individuals, rather than in patients with active asthma. Thus, roxithromycin-induced apoptosis of *D. farinae*-stimulated cells mimics the increase of apoptosis in patients with remission who are tolerant of mite antigen.

This increase of apoptosis was not induced by other antibiotics, including cefazolin and ampicillin, indicating that roxithromycin was specifically involved in the augmentation of apoptosis.

Fas ligand expression by *D. farinae*-stimulated cells, but not unstimulated or phytohemagglutinin-stimulated cells, was significantly increased after treatment with 1 μg/ml roxithromycin when compared to no treatment. These results show clearly that roxithromycin is involved in Fas ligand expression and *D. farinae*-induced apoptosis. In contrast, Fas receptor expression was not changed by simulation with *D. farinae* or phytohemagglutinin. Bcl-2 expression was reduced by treatment with roxithromycin in the case of unstimulated as well as *D. farinae*-stimulated cells. These results suggest that the impaired apoptotic response to *D. farinae* stimulation in patients with mite-sensitive asthma was restored by treatment with roxithromycin. Induction of Fas/Fas ligand and reduced Bcl-2 expression were involved in the increase of apoptosis.

Initiation and augmentation of interleukin-4 production by activated cord blood T cells (Shu et al., 1994) implies that infants exposed to an allergen, such as *Dermatophagoides* antigen, may develop allergic diseases that are mediated by interleukin-12 secreted from infected cells, including phagocytic cells, natural killer cells, and T cells when recurrent infection with microorganisms occurs early in life. Indeed, high-dose interleukin-12 did induce interleukin-4 production by *D. farinae*-activated PBM cells from pediatric patients allergic to mite antigen (Noma and Yoshizawa, 1999), which is similar to what is observed in neonates (Shu et al., 1994). Interleukin-12 presumably initiates the disease process, or enhances the sensitivity of genetically susceptible individuals. Interleukin-12 shows a different effect on antigen-induced interleukin-4 and interferon-gamma production, depending on whether a low dose or

high dose is added. Infants exposed to an allergen tend to develop allergic diseases, such as asthma, early in life when they suffer from recurrent infection with microorganisms that induce interleukin-12 production by cells such as phagocytic cells. Respiratory syncytial virus (RSV) causes respiratory tract infection in neonates (Henderson and Clyde, 1979). Since some patients suffer from recurrent wheezing after RSV infection, attention has been directed to the progression of this disease to asthma. After RSV infection, lymphocytes acquire specific susceptibility to *D. farinae*, a mite antigen, and food antigens (particularly ovalbumin), probably by secreting cytokines like interleukin-4, which is able to induce Th2 or Th0 type helper T cells (Noma et al., 1996). Activation of helper T cells (Th1, Th2, or Th0) (Mosmann and Coffman, 1989) involved in allergic reactions is likely to occur after RSV infection, resulting in increased secretion of cytokines such as interleukin-2, interleukin-3, GM-CSF, interleukin-4, interleukin-5, interleukin-6, and interferon-gamma, with a consequent imbalance being likely. Production of interleukin-12 by RSV-infected cells lead to the production of these cytokines, because high (but not low) doses of interleukin-12 can induce interleukin-4 and IgE production in *D. farinae*-activated lymphocytes from children with mite-sensitive asthma (Noma and Yoshizawa, 1999). If infants with an allergic predisposition, whose lymphocytes are activated by allergens, are infected with a virus such as RSV, the high levels of interleukin-12 produced by RSV-infected cells may cause atopic diseases, particularly after recurrent exposure to allergens. Roxithromycin may be an effective treatment for asthma, especially when patients have concomitant viral infections such as RSV infection. The action of roxithromycin to augment apoptosis of RSV-activated lymphocytes, which was suggested by the results of this study, may prevent the initiation or progression of allergic diseases.

Thus, pediatric asthma patients who are infected with viruses like RSV may be effectively treated with roxithromycin. The present observations on the immunological effects of roxithromycin may help to shed light on the pathogenesis, and potentially the prevention, of allergic diseases in infants.

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