

Sephadex G-200-Induced Eosinophil Infiltration into Airways in Non-sensitized and Sensitized Guinea Pigs, and Responsiveness of the Cells to Stimuli In Vitro

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Received October 17, 1995 Accepted February 1, 1996

ABSTRACT—Eosinophils are thought to be one of the pathophysiologically pivotal cells in atopic-type inflammation. In the present experiments, the in vitro responsiveness to stimuli of eosinophils, which had infiltrated into the airway following intravenous administration of Sephadex G-200 (Sephadex), was mainly studied in non-sensitized and [antigen + Al(OH)₃]-sensitized guinea pigs. In sensitized, Sephadex-treated guinea pigs, a large number of eosinophils were found in the bronchoalveolar lavage fluid, whereas a much smaller number of cells were recovered in either non-sensitized or sensitized, Sephadex-untreated animals and a smaller number were recovered in non-sensitized Sephadex-treated animals. The eosinophils from non-sensitized Sephadex-treated guinea pigs released superoxide anion ($\cdot\text{O}_2^-$) and thromboxane (TX) B₂ in response to platelet-activating factor (PAF), leukotriene B₄ and Ca ionophore A23187. Either spontaneous or PAF-induced $\cdot\text{O}_2^-$ generation from eosinophils of sensitized, Sephadex-treated guinea pigs was significantly greater than that from non-sensitized animals, while TXB₂ release stimulated by any of the above stimuli was not further enhanced by sensitization. These results indicate that active sensitization can change some eosinophil functions and that the functionally altered cells could play a pathophysiological role in atopic inflammation.

Keywords: Eosinophil, Sensitization, Sephadex, Superoxide anion, Thromboxane B₂

In patients with allergic bronchial asthma, an increased number of eosinophils have been found in the circulating blood (1, 2), sputum (2) and bronchial airways (3, 4). Eosinophils migrating to the inflammatory site are most likely further activated by stimulation with concomitantly occurring biological reactive substances to release potent biologically active mediators. Among these, in particular, granule proteins (major basic protein (5), eosinophil cationic protein (6), eosinophil-derived neurotoxin (7) and eosinophil peroxidase (8)), active oxygen species such as superoxide anion ($\cdot\text{O}_2^-$) (9–11) and lipid mediators including thromboxane (TX) A₂ (12–14) and leukotriene (LT) C₄ (15) are considered to aggravate asthma; for example, by injuring or removing the epithelial cells (16–18) and causing airway smooth muscle contraction (19, 20). Although our understanding of the pathophysiological roles of eosinophils in atopic diseases, especially allergic bronchial asthma, is greater than ever, there is

still much left to elucidate.

For studies on the pathophysiological roles of eosinophils in atopic diseases, guinea pigs have often served as a good source of cells among experimental animals. The majority of guinea pig eosinophil experiments have employed cells that migrated into the peritoneal cavity following intraperitoneal injection of a polypeptide or some proteins repeatedly for several days (21, 22). On the other hand, Maghni et al. (23) reported that a single intravenous administration of various sizes of Sephadex G type beads causes eosinophils and neutrophils to accumulate in the airway for a relatively short time in normal guinea pigs.

In the present study, we examined the characteristics of eosinophils that had been recovered from the bronchoalveolar lavage fluid (BALF) of non-sensitized and actively sensitized guinea pigs treated with Sephadex G-200, in terms of their density and responsiveness to various stimuli.

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MATERIALS AND METHODS

Reagents

Reagents and their sources were as follows: Sephadex G-200 (Sephadex, Superfine) and Percoll (Pharmacia, Uppsala, Sweden); bovine serum albumin (BSA), ovalbumin (OA), LTB₄, superoxide dismutase (Cu,Zn-type, from bovine erythrocyte: SOD) and histamine dihydrochloride (Wako Pure Chem., Osaka); LTD₄ (a gift from Ono Pharm., Co., Osaka); Ca ionophore A23187 (A23187; Calbiochem-Novabiochem, La Jolla, CA, USA); substance P (SP), neurokinin A (NKA) and endothelin (ET)-1 (Peptide Institute, Osaka); U46619 (Funakoshi, Tokyo); cytochrome *c* (from horse heart; Sigma Chem., St. Louis, MO, USA); TXB₂ enzyme immunoassay (EIA) kit (Cayman Chem., Ann Arbor, MI, USA); and trypan blue, May-Grünwald stain solution and Giemsa stain solution (Nacalai Tesque, Kyoto).

C-18 platelet-activating factor (PAF) was kindly donated by Dr. Y. Ashida of Takeda Chem., Ind., Osaka.

The other reagents used were the highest grade commercially available.

Animals

Male 3-week-old Hartley guinea pigs weighing 230–250 g were purchased from Japan SLC (Hamamatsu).

Sensitization

Guinea pigs were sensitized by intraperitoneal injection with 10 µg OA/mg Al(OH)₃/animal/time once every 2 weeks for a total of 14 times (24). The sensitized animal showed that the 4-hr and 7-day passive cutaneous anaphylaxis titer ranged from 1 : 400 to 1 : 2,000 when assessed using the serum, and it was used for eosinophil examinations 7–14 days after the last sensitization.

Sephadex-induced eosinophil infiltration into airways

Eosinophil infiltration into the airway was induced by Sephadex according to the method of Maghni et al. (23). In brief, Sephadex beads suspended in physiological saline at 10 mg/0.8 ml/kg were administered intravenously to a non-sensitized or sensitized guinea pig. Eighteen hours later, the animal was exsanguinated from the femoral artery under pentobarbital anesthesia. After perfusion of the lung with Ca²⁺-free BSA-containing Tyrode's solution (Ca²⁺-free Tyrode's solution) via the pulmonary artery, bronchoalveolar lavage (BAL) was performed with Ca²⁺-free Tyrode's solution (10 ml × 5). The recovered fluids were washed twice with Ca²⁺-free Tyrode's solution. The total cell number and viability were determined by trypan blue and the differential cell counts by May-Grünwald-Giemsa staining. BAL was also adapted to non-sensitized and sensitized guinea pigs

without treatment of Sephadex.

Purification of eosinophils

Purification of alveolar eosinophils was performed by the method of Hirata et al. (13). After hypotonic treatment and incubation in a polystyrene culture flask (225 cm²) at 37°C for 1 hr in 5% CO₂ and 95% air, suspended non-adherent BAL cells were stratified on discontinuous (50%, 60% and 70%) Percoll layers. Following centrifugation at 360 × *g* for 30 min at 4°C, eosinophils were recovered from the bottom of the centrifuge tube. Cell viability and eosinophil purity were more than 95% and 92 ± 0.6% (*n* = 15), respectively. Other cells were neutrophils (5 ± 0.6%) and mononuclear cells (MNC) (3 ± 0.3%). The suspended, purified eosinophils were used for the experiments of superoxide anion ($\cdot\text{O}_2^-$) generation and TXB₂ release.

For the assessment of eosinophil density distribution by centrifugation, 50%, 60%, 70% and 80% Percoll solutions corresponding to densities of 1.063, 1.075, 1.088 and 1.100, respectively, were used.

$\cdot\text{O}_2^-$ production

Production of $\cdot\text{O}_2^-$ was measured by the reductive method using cytochrome *c* (25). Briefly, 100 µM cytochrome *c* and various concentrations of stimuli were mixed in Tyrode's solution in the presence or absence of 158 U/ml of SOD. Following preincubation at 37°C for 5 min, the reaction was started by the addition of an equal volume of purified eosinophils (2 × 10⁶ cells/ml) to the mixture. At 15, 30, 60, 120 and 180 min, the absorbances at 540 and 550 nm of the reaction solution were measured. $\cdot\text{O}_2^-$ production was calculated from the difference in the absorbance between the 2 wavelengths with an extinction coefficient of 19.1 × 10³/M/cm as nmol of cytochrome *c* reduced/10⁶ eosinophils.

TXB₂ release

After preincubation at 37°C for 5 min, to the purified eosinophils (1.1 × 10⁶ cells/ml) were added various concentrations of stimuli at 1/10 volumes, and the reaction was allowed to proceed at 37°C for 15 min. The reaction was stopped by cooling in ice-water, followed by centrifugation at 1,700 × *g* for 15 min at 4°C. The resultant supernatant was stored at –80°C until assay of TXB₂. TXB₂ in the supernatant was measured by EIA and expressed as pg/10⁶ eosinophils.

Statistical analyses

Statistical analyses were performed by one-way analysis of variance (ANOVA). If a significant difference was detected, the individual group difference was determined by Bonferroni's multiple test. A probability value (*P*) of

Table 1. Sephadex G-200 (Sephadex)-induced leukocyte infiltrations into airways in non-sensitized and actively sensitized guinea pigs

Sephadex-treatment	(n)	Cell number, $\times 10^6$ cells/animal (% of total cells)				
		Total cells	Mononuclear cells	Eosinophils	Neutrophils	Others
Non-sensitized	— (8)	52.4 \pm 12.2 (100)	47.8 \pm 10.4 (92.6 \pm 1.5)	3.4 \pm 1.8 (5.7 \pm 1.4)	1.1 \pm 0.7 (1.3 \pm 0.8)	0.1 \pm 0.1 (0.4 \pm 0.3)
	+ (5)	145.0 \pm 15.9 ^{aa} (100)	76.3 \pm 9.1 (52.4 \pm 2.1)	32.9 \pm 5.5 ^{aa} (23.1 \pm 3.3)	35.6 \pm 5.1 ^{aa} (24.3 \pm 1.5)	0.3 \pm 0.2 (0.2 \pm 0.1)
Sensitized	— (4)	121.6 \pm 17.0 (100)	115.9 \pm 16.2 ^{aa} (95.5 \pm 2.3)	4.2 \pm 2.9 (3.4 \pm 2.2)	1.4 \pm 0.4 (1.1 \pm 0.2)	0.1 \pm 0.1 (0.1 \pm 0.1)
	+ (3)	289.8 \pm 35.8 ^{bb,cc} (100)	162.8 \pm 21.9 ^{bb} (56.1 \pm 1.9)	67.7 \pm 3.5 ^{bb,cc} (23.9 \pm 2.0)	58.7 \pm 13.1 ^{b,cc} (19.8 \pm 2.9)	0.4 \pm 0.3 (0.1 \pm 0.1)

Bronchoalveolar lavage was performed 18 hr after the Sephadex treatment (10 mg/kg, i.v.). Each value represents the mean \pm S.E. of the No. of animals shown in parentheses. ^{aa}P < 0.01 vs non-sensitized Sephadex non-treated group. ^{bb}P < 0.05, ^{bb}P < 0.01 vs non-sensitized Sephadex-treated group. ^{cc}P < 0.01 vs sensitized Sephadex non-treated group.

less than 0.05 was considered to be statistically significant.

RESULTS

Sephadex-induced eosinophil infiltration into airways

Table 1 shows the number and percentage of sorted leukocytes recovered in BALF in non-sensitized and sensitized guinea pigs by the treatment with or without

Sephadex.

In non-sensitized animals, 145.0×10^6 total cells/animal were found in the Sephadex-treated animal, which was 2.8 times greater than the number in the non-treated animal (52.4×10^6 total cells/animal). Both the number and percentage of eosinophils were increased by the treatment, reaching levels approx. 10 and 4 times, respectively, more than those of the non-treated animal. In terms of the percentage, neutrophils and eosinophils were

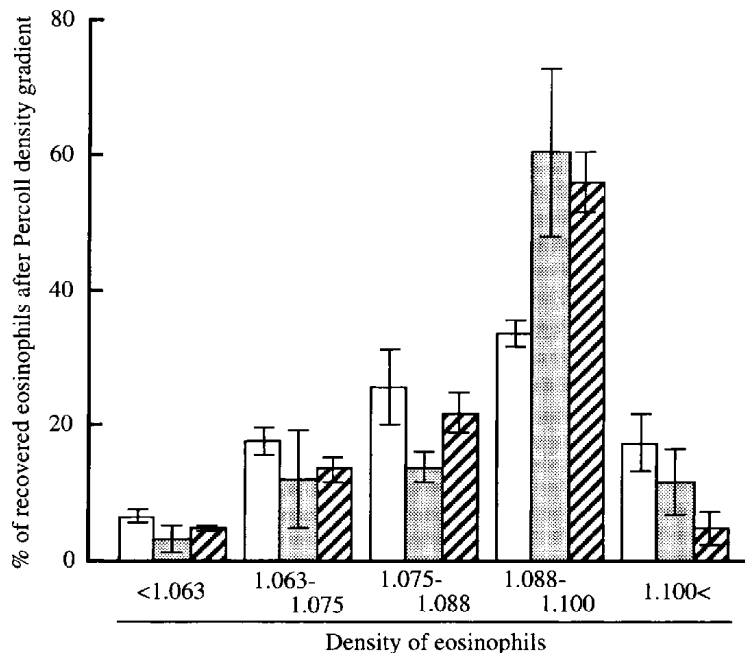


Fig. 1. Density distributions of bronchoalveolar eosinophils in non-sensitized and actively sensitized guinea pigs treated with or without Sephadex G-200 (Sephadex). Bronchoalveolar lavage was performed 18 hr after the Sephadex treatment (10 mg/kg, i.v.). □: non-sensitized, non-treated; ▤: non-sensitized, Sephadex-treated; ▨: sensitized, non-treated; ■: sensitized, Sephadex-treated. Each column represents the mean \pm S.E. of 3–5 of animals.

increased by the treatment at the expense of a decrease in MNC. On the other hand, in the sensitized guinea pig not treated with Sephadex, the total cells were comparable to those in the non-sensitized Sephadex-treated animal, although the majority of the cells were MNC with percentages of the respective leukocytes similar to those in the non-sensitized animal not treated with Sephadex. By Sephadex treatment, eosinophil migration into the airway was induced, with the number being almost double that of the non-sensitized, Sephadex-treated animals and 16 times greater than the sensitized Sephadex-non-treated animal. Although an increased number of total cells, MNC, eosinophils and neutrophils were observed in the sensitized Sephadex-treated animal, the percentages of the respective leukocytes were almost equivalent to those of the non-sensitized Sephadex-treated animal.

Density distributions of eosinophils collected from non-sensitized and sensitized guinea pigs treated with or without Sephadex

The density distributions of eosinophils collected by BAL from non-sensitized and sensitized guinea pigs treated with or without Sephadex were examined using Percoll discontinuous layers and centrifugation. No large differences in the density distribution were found in any of these groups; a considerable number of eosinophils were located at the interface between the 70% and 80% Percoll layers, corresponding to the density of

1.088–1.100 (Fig. 1).

Spontaneous and stimulus-induced $\cdot\text{O}_2^-$ production of eosinophils from non-sensitized and sensitized guinea pigs treated with Sephadex

PAF and LTB_4 concentration-dependently induced $\cdot\text{O}_2^-$ production from the purified eosinophils in non-sensitized guinea pigs, which reached a plateau at 60 min of incubation and was completely reversed by pretreatment with 76 U/ml SOD. Three hundred nanomolar A23187 also potently and time-dependently induced $\cdot\text{O}_2^-$ production and the increment was still recognized at 180 min of incubation, whereas 100 nM of the agent produced only modest stimulation (Fig. 2).

The time course experiments revealed that spontaneous $\cdot\text{O}_2^-$ production from the eosinophils in sensitized guinea pigs increased significantly more than that in the non-sensitized ones (Fig. 3). In addition, PAF-stimulated $\cdot\text{O}_2^-$ production from the eosinophils in sensitized guinea pigs was also obviously greater than that in non-sensitized animals (Fig. 4). Yet, slight enhancement and no enhancement of the production induced by LTB_4 and A23187, respectively, were observed (Fig. 4 and Table 2).

Histamine at 10 and 100 μM , ET-1 and U46619 at 0.1 and 1 μM , SP and NKA at 1 and 10 μM , and LTD_4 at 0.01 and 0.1 μM caused little or no enhancement of $\cdot\text{O}_2^-$ production. The sensitization also did not affect these reactions significantly (Table 2).

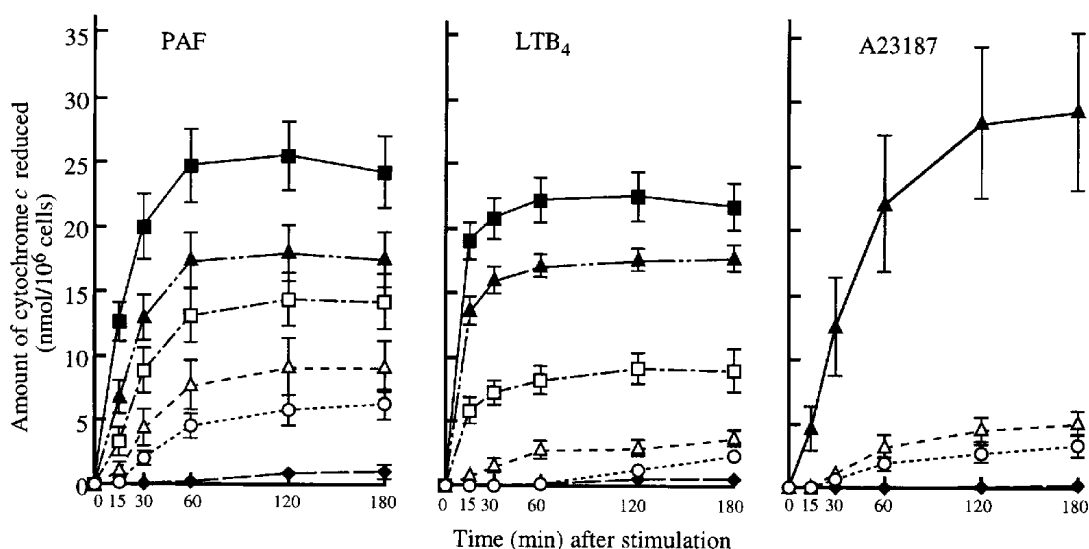


Fig. 2. Time courses of platelet-activating factor (PAF, left panel)-, leukotriene (LT) B_4 (middle panel)- and Ca ionophore A23187 (A23187, right panel)-induced superoxide anion production of bronchoalveolar eosinophils in non-sensitized guinea pigs treated with Sephadex G-200. Eosinophils were incubated with 50 μM cytochrome c in the absence or presence of various stimuli at 37°C for the indicated time. Left panel (PAF): \circ , spontaneous (Spon); \triangle , 1 nM; \square , 10 nM; \blacktriangle , 100 nM; \blacksquare , 1000 nM; \blacklozenge , 1000 nM + superoxide dismutase (SOD) 76 U/ml. Middle panel (LTB_4): \circ , Spon; \triangle , 10 nM; \square , 30 nM; \blacktriangle , 100 nM; \blacksquare , 300 nM; \blacklozenge , 300 nM + SOD 76 U/ml. Right panel (A23187): \circ , Spon; \triangle , 100 nM; \blacktriangle , 300 nM; \blacklozenge , 300 nM + SOD 76 U/ml. Each point represents the mean \pm S.E. of 3–7 experiments.

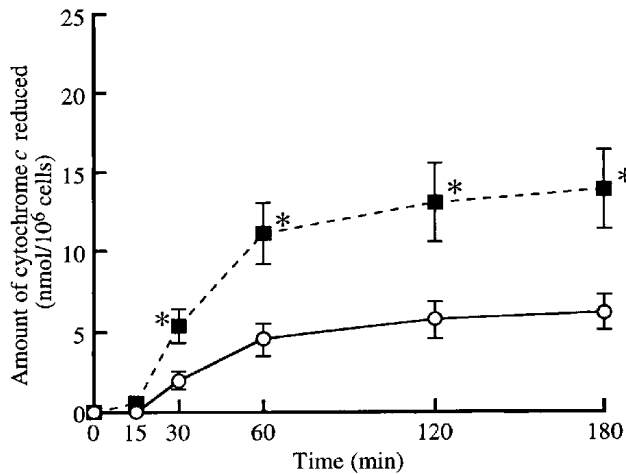


Fig. 3. Time courses of spontaneous superoxide anion production of bronchoalveolar eosinophils in non-sensitized (○) and actively sensitized guinea pigs (■) treated with Sephadex G-200. Eosinophils were incubated with 50 μ M cytochrome *c* at 37°C for the indicated time. Each point represents the mean \pm S.E. of 7 and 3 experiments for non-sensitized and sensitized animals, respectively. * $P < 0.05$ vs non-sensitized group.

On the other hand, eosinophils from sensitized guinea pigs showed no enhancement of $\cdot\text{O}_2^-$ production by the antigen challenge (Table 2).

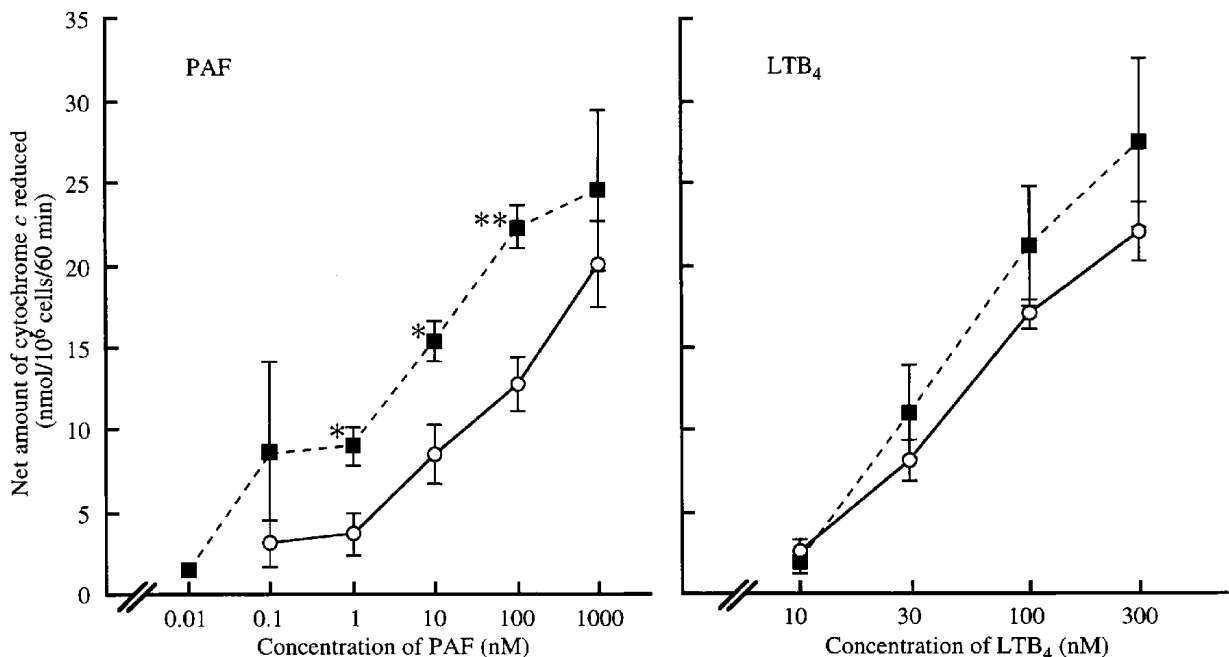


Fig. 4. Concentration-response curves of platelet-activating factor (PAF, left panel)- and leukotriene (LT) B₄ (right panel)-induced superoxide anion production of bronchoalveolar eosinophils in non-sensitized (○) and actively sensitized guinea pigs (■) treated with Sephadex G-200. Eosinophils were incubated with 50 μ M cytochrome *c* at 37°C for 60 min. Each point represents the mean \pm S.E. of 5–7 and 3 experiments for non-sensitized and sensitized guinea pigs, respectively. * $P < 0.05$, ** $P < 0.01$ vs non-sensitized group.

Stimulus-induced TXB₂ release of eosinophils from non-sensitized and sensitized guinea pigs treated with Sephadex

Similar to the results of $\cdot\text{O}_2^-$ production, PAF and LTB₄ concentration-dependently enhanced TXB₂ release from the eosinophils in non-sensitized guinea pigs, although the potencies to induce the release were different from each other. Sensitization did not alter TXB₂ release from the cells induced by either PAF and LTB₄ (Fig. 5).

A23187 concentration-dependently induced TXB₂ release from the eosinophils in non-sensitized guinea pigs with a marked release of the prostanoid at 1000 nM of the compound. Modest amounts of TXB₂ were released from the non-sensitized guinea pig eosinophils in a concentration-dependent fashion by histamine at 10 and 100 μ M, ET-1 at 0.1 and 1 μ M, and LTD₄ at 0.01 and 0.1 μ M. Neither SP nor NKA at 1 and 10 μ M induced the TXB₂ release. These reactions were hardly influenced by the sensitization except for the tendency of A23187-induced release to be (Table 2).

As in the case of $\cdot\text{O}_2^-$ production, eosinophils from sensitized guinea pigs did not release TXB₂ in response to antigen stimulation (Table 2).

Table 2. Various stimuli- or antigen (ovalbumin)-induced superoxide anion production and thromboxane (TX) B₂ release from bronchoalveolar eosinophils is non-sensitized and actively sensitized guinea pigs treated with Sephadex G-200

Stimulus	Conc.	Net amounts of cytochrome <i>c</i> reduced (nmol/10 ⁶ cells/180 min)		Net amounts of TXB ₂ released (pg/10 ⁶ cells)	
		Non-sensitized	Sensitized	Non-sensitized	Sensitized
Ca ionophore A23187	100 nM	1.8±0.2	1.1±1.1	—	—
	300 nM	26.0±5.4	27.7±3.0	217.4±145.0	1.0±0.6
	1000 nM	—	—	4379.9±839.7	1888.8±870.2
Histamine	10000 nM	0.4±0.2	0.8±0.4	11.7±11.5	9.3±5.6
	100000 nM	0.5±0.5	2.0±0.7	24.0±23.6	2.8±2.8
Endothelin-1	100 nM	0.1±0.1	1.7±1.7	4.0±3.7	8.5±6.8
	1000 nM	0.2±0.2	2.3±1.7	15.0±12.6	2.1±2.1
Substance P	1000 nM	0.6±0.4	0.4±0.4	0.4±0.2	0.1±0.1
	10000 nM	0.3±0.2	1.6±1.6	0±0	0.7±0.7
Neurokinin A	1000 nM	0.3±0.3	0.1±0.1	1.5±0.5	7.9±7.9
	10000 nM	0.1±0.1	0±0	0.6±0.4	1.6±0.9
Leukotriene D ₄	10 nM	1.1±0.7	0.7±0.4	1.7±1.7	2.8±1.6
	100 nM	0.1±0.1	0.5±0.5	10.9±1.1	8.9±2.8
U46619	100 nM	0.7±0.6	1.2±1.0	—	—
	1000 nM	0.4±0.4	0.9±0.9	—	—
Antigen (ovalbumin)	1 µg/ml	—	0±0	—	2.7±1.5
	10 µg/ml	—	0±0	—	0.9±0.9
	100 µg/ml	—	3.7±3.7	—	0±0

Spontaneous amounts of cytochrome *c* reduced and TXB₂ release of non-sensitized and sensitized guinea pig eosinophils were 2.4±0.6 and 10.3±2.6 nmol/10⁶ cells/180 min and 20.0±7.5 and 9.3±0.9 pg/10⁶ cells, respectively. Each value represents the mean±S.E. of 3 or 4 experiments. —: not done.

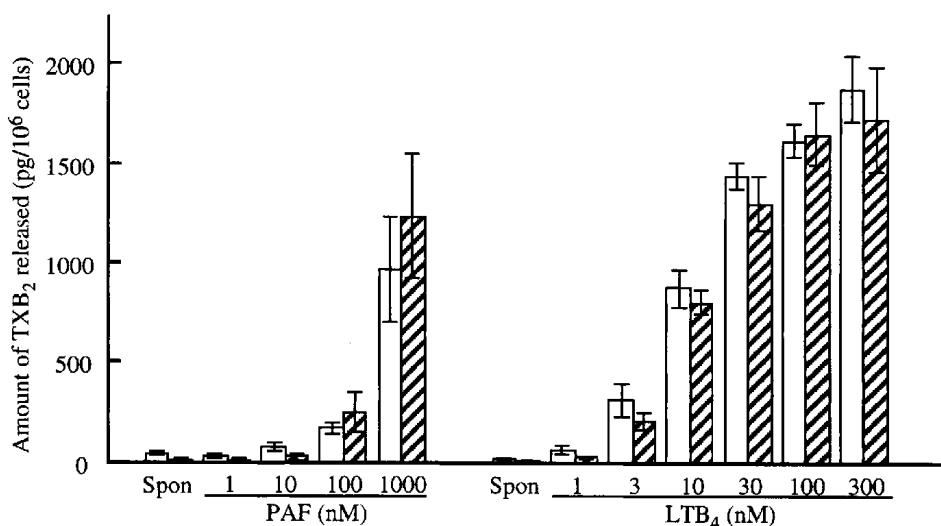


Fig. 5. Platelet-activating factor (PAF)- and leukotriene (LT) B₄-induced thromboxane (TX) B₂ release from bronchoalveolar eosinophils is non-sensitized (□) and actively sensitized guinea pigs (▨) treated with Sephadex G-200. Eosinophils were incubated for 15 min in the absence or presence of the stimuli at 37°C. Each column represents the mean±S.E. of 3–6 experiments.

DISCUSSION

Maghni et al. (23) reported that intravenous administration of various sizes of Sephadex G type beads causes

eosinophils and neutrophils to accumulate into the airway in normal guinea pigs and that these leukocytes infiltrate outside from capillary blood vessels plugged by Sephadex beads. Reexamination following their method was firstly

performed in the present study, and good results were obtained by the use of any type of Sephadex G beads in accordance with their report. Among these, Sephadex G-200 (Sephadex) was chosen as an excellent agent in terms of the degree of eosinophil accumulation in the airway, lethality and injury to the lung. Consequently, intravenous administration of Sephadex markedly increased the number of both cell types in BALF, which reached more than 9 times those in untreated guinea pigs. After sensitization by intraperitoneal injection with antigen absorbed on aluminum hydroxide gel, the number of MNC in BALF from guinea pigs without treatment of Sephadex increased significantly. Although neither eosinophils nor neutrophils in BALF were augmented by the sensitization in animals not treated with Sephadex, migration of these kinds of cells into the airway may have been caused by the treatment with Sephadex. This can be derived from the increased number of eosinophils and neutrophils as well as MNC in the blood in the sensitized state. In addition, it is speculated that the active sensitization can enhance the expression of some adhesion molecules on the eosinophil membrane because it has been reported that expression of CD11b/CD18 on the cell membrane increases when the cells are activated (12, 26, 27).

Many investigators have pointed out that there is a shift in the type of eosinophils in the blood and BALF from patients with allergic bronchial asthma to hypodense from normodense (28–30). Furthermore, it has been reported that when actively sensitized guinea pigs were challenged by inhalation of antigen and the eosinophils, which migrated into the airway, were assessed, the cells had become slightly hypodense (31). In the present experiments, the eosinophils recovered in BALF appeared to be normodense regardless of whether or not they had been sensitized or treated with Sephadex because the distribution of eosinophils as seen by density gradient centrifugation seemed similar to that of the cells from the blood of normal human subjects (28, 29).

Coëffier et al. (32) demonstrated that when actively sensitized guinea pigs were challenged by instillation of antigen into the nasal cavity, a significant increment of eosinophils in BALF and enhanced chemotaxis of these cells by stimulation with PAF were induced, and the latter phenomenon was inhibited by pretreatment with anti-murine interleukin-5 (IL-5) antibody. Furthermore, it has been shown that incubation of guinea pig and human eosinophils with IL-5 gave the eosinophils the ability to enhance biological responses to PAF in vitro (11, 33–35). In the present experiments, two parameters, TXB₂ release and $\cdot\text{O}_2^-$ production, were chosen as indexes of the responsiveness of eosinophils to stimuli because the guinea pig eosinophil is able to form these mediators in

large quantities (10, 13, 14). Interestingly, the purified eosinophils from the sensitized guinea pig did not show enhancement of spontaneous release of TXB₂, whereas spontaneous $\cdot\text{O}_2^-$ production was significantly increased compared to that from non-sensitized guinea pigs. Consequently, concentration-dependent $\cdot\text{O}_2^-$ production induced by PAF was significantly enhanced in sensitized guinea pigs, while TXB₂ release induced by any stimuli was not different from that of non-sensitized animals. The enhanced $\cdot\text{O}_2^-$ production might be attributable to augmented expression of a membrane protein, cytochrome *b*₅₅₈, which is a constituent of the respiratory burst oxidase, since it has been suggested that eosinophil-like erythroleukemic cells (HL-60 cells) induce elevation of respiratory burst activity via enhancement of cytochrome expression during incubation with tumor necrosis factor or interferon- γ for several days (36).

Although eosinophils of humans and guinea pigs have been reported to acquire various biologically positive responses to stimuli within a relatively short time after the treatments of the cells or animals with cytokines or antigen either in vitro or in vivo as described above, the present eosinophils from sensitized guinea pigs showed only spontaneously enhanced and enhanced PAF-induced $\cdot\text{O}_2^-$ generation. The conditions of the present experiments differ considerably from those described above: 1) Although guinea pigs were highly sensitized by repeated intraperitoneal injection with antigen, the in vitro experiments were performed at a relatively long time after the last sensitization; 2) it is unlikely that the cells participating in anaphylaxis are stimulated at the repeated antigen sensitization, as the antigen was almost completely absorbed onto aluminum hydroxide gel, so that the cells are not supposed to be significantly influenced by antigen stimulation. In other words, the eosinophils are presumably prepared to generate $\cdot\text{O}_2^-$ for stimulation with various stimuli, but not to stimulate the arachidonate pathway under the influence of putative cytokines derived from immune-competent cells. Thus, if anaphylaxis is provoked by challenge with an antigen without aluminum hydroxide gel, the eosinophils of the present sensitized guinea pig may be set in more activated conditions showing a variety of responses to stimulation.

Although significant levels of cytophilic antibodies, IgE and γ_1 , in the blood of the sensitized guinea pigs were detected and human eosinophils are believed to possess the high and low affinity receptors for IgE on their membrane surfaces (37, 38), the cells showed neither TXB₂ nor $\cdot\text{O}_2^-$ generation in response to antigen. Thus, guinea pig eosinophils may not have such receptors or may require some additional biological modulators or other cells to become activated.

Taken together, these results indicate that the sensitized

state without antigen challenge activates eosinophils to some extent.

Acknowledgments

We express our sincere gratitude to K. Ikoma, E. Kitaguchi and Y. Shindo for their technical assistance.

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