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Detection of activated eosinophils in nasal polyps of an aspirin-induced asthma patient*

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SUMMARY

Aspirin-induced asthma (AIA) is frequently accompanied by nasal polyps. Eosinophil infiltration is a characteristic feature of nasal polyps associated with AIA. Even though steroids are well known to be effective on managing AIA and its nasal polyps, histochemical examinations after steroid therapy and at recurrence, involving eosinophil infiltration of nasal polyps, have been less studied. To know the histochemical effects of steroid treatment on eosinophil accumulation in nasal polyps of AIA and the histochemical feature of a recurring polyp and to detect distributional differences between storage and secreted forms of eosinophil cationic proteins, we carried out immunocytochemical labelling with antibodies against EG1 (recognizing resting and activated eosinophils) and EG2 (recognizing only activated eosinophils), and determined eosinophil infiltration in nasal polyps that were obtained before and after steroid treatment, and at recurrence of polyps. A large number of eosinophils in AIA polyps were found before steroid treatment and at recurrence, and they were predominantly composed of activated eosinophils (EG2-positive). In contrast, eosinophil infiltration was rare in polyps obtained immediately after steroid treatment. This finding suggests that eosinophil infiltration may be associated with nasal polyp formation in AIA, and that activation of eosinophils plays an important role in accumulation of eosinophils and polyp formation beginning with the initial stage.

Key words: activated eosinophil, nasal polyp, aspirin-induced asthma, steroid treatment

INTRODUCTION

Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) may precipitate asthma attacks. This distinct clinical syndrome is called aspirin-induced asthma (AIA) (Szczeklik, 1992). Since Gilbert (1911) first documented an acute asthmatic attack caused by an idiosyncratic reaction to aspirin, AIA has become a relatively common disease. It has been observed in 10% of adults with asthma (Szczeklik, 1992). The cyclooxygenase theory has been accepted as the most likely explanation of AIA pathogenesis (Szczeklik, 1992). Aspirin or NSAIDs inhibit cyclooxygenase in the arachidonic acid metabolism pathway, and this inhibition appears to set off a chain reaction leading to asthma attacks in aspirin-intolerant patients (Probst et al. 1992; Szczeklik, 1992).

It is generally accepted that the triad of AIA comprises aspirin idiosyncrasy, asthma, and nasal polyps. Nasal polyps of AIA patients characteristically contain a eosinophil infiltrate (Ogawa, 1986; Jankowski et al., 1989; Jankowski, 1996), and this infiltration is assumed to be associated with nasal polyp formation (Holopainen et al., 1979; Yamashita et al., 1989; Yoshimi et al., 1993; Jankowski, 1996).

Steroids are widely used to treat patients with allergic and inflammatory airway disorders involving AIA. However, a few histochemical and immunocytochemical studies have been reported which examine the effects of steroid treatment on eosinophil accumulation in the nasal polyps and the re-infiltration of eosinophils in a recurring polyp associated with AIA. We encountered a case of an AIA patient who received steroid treatment for a severe asthma attack after a nasal polypectomy, and then had a recurring polyp 5 months later.

We present the immunocytochemical comparison of eosinophil infiltration found in the nasal polyps at surgery, after steroid treatment, and at recurrence, using antibodies to EG1 and EG2, and discuss eosinophil infiltration in nasal polyps associated with AIA.

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MATERIALS AND METHODS

Subject

A 49-year-old man had a severe asthma attack after ingesting aspirin in 1994, and was diagnosed on the basis of clinical history as having AIA. He had suffered from nasal stuffiness caused by nasal polyps, and he underwent a nasal polypectomy under local anaesthesia in 1995 (specimen-1). Remnant polyps gradually increased in size, although the patient received local administration of beclomethasone (50 μg twice a day) after surgery. In Spring 1996, the patient had a severe asthma attack and was transferred to our hospital with his status listed as dead on arrival. Immediately he received intravenous drip infusion of hydrocortisone (1200 mg) and other lifesaving treatments, and he revived. Subsequently, he was given hydrocortisone (1200 mg) for 2 days and prednisolone (30 mg) for 7 days. His nasal condition improved dramatically. The remnant polyps disappeared, and x-ray examination showed clear sinuses. Some edematous and polypoid mucosa of the middle turbinate and bulla were removed to enlarge the middle meatus (specimens-2). Five months later, a small polyp was observed in the middle meatus, and it was removed under local anaesthesia (specimen-3).

Tissue preparation

The specimens were fixed in 10% formaldehyde. After being washed in phosphate buffered saline (PBS), they were dehydrated by an ascending series of ethanol, and then embedded in paraffin. Sections cut at 5 μm thickness were placed onto subbed slides and air-dried. The paraffin was removed from the sections by immersing the slides in clear xylene. The sections were then rehydrated through a descending series of ethanol to water and processed for immunocytochemical labelling.

Immunocytochemical labelling

Eosinophils were confirmed on the sections by a Histofine SAB-PO(M) stain kit (Nichirei, Tokyo, Japan), and by 2 mouse monoclonal antibodies to human eosinophil cationic protein (ECP) (Kabi Pharmacia, Uppsala, Sweden): one was an antibody against EG1 that recognizes both resting and activated eosinophils, and the other an antibody against EG2 that recognizes only activated eosinophils (Tai et al., 1984; Moqbel et al., 1992). The sections were preincubated with 3% H_2O_2 in methanol for 10 min at room temperature and washed in PBS for 15 min. Then the sections were reacted with blocking serum (10% rabbit serum) for 10 min, primary antibodies against EG1 and EG2 at a dilution of 1:100 in PBS overnight at 4°C, washed in PBS for 15 min, blocking serum for 10 min again, biotinylated anti-mouse secondary antibodies (10 μg/ml) for 20 min, and streptavidin-peroxidase for 10 min at room temperature. The immunocytochemical labelling was visualized by 3, 3'-diaminobenzidine, and the sections were counterstained with methylene blue. They were subsequently rinsed in distilled water, dehydrated in an ascending series of ethanol and clear xylene, and dropped His-

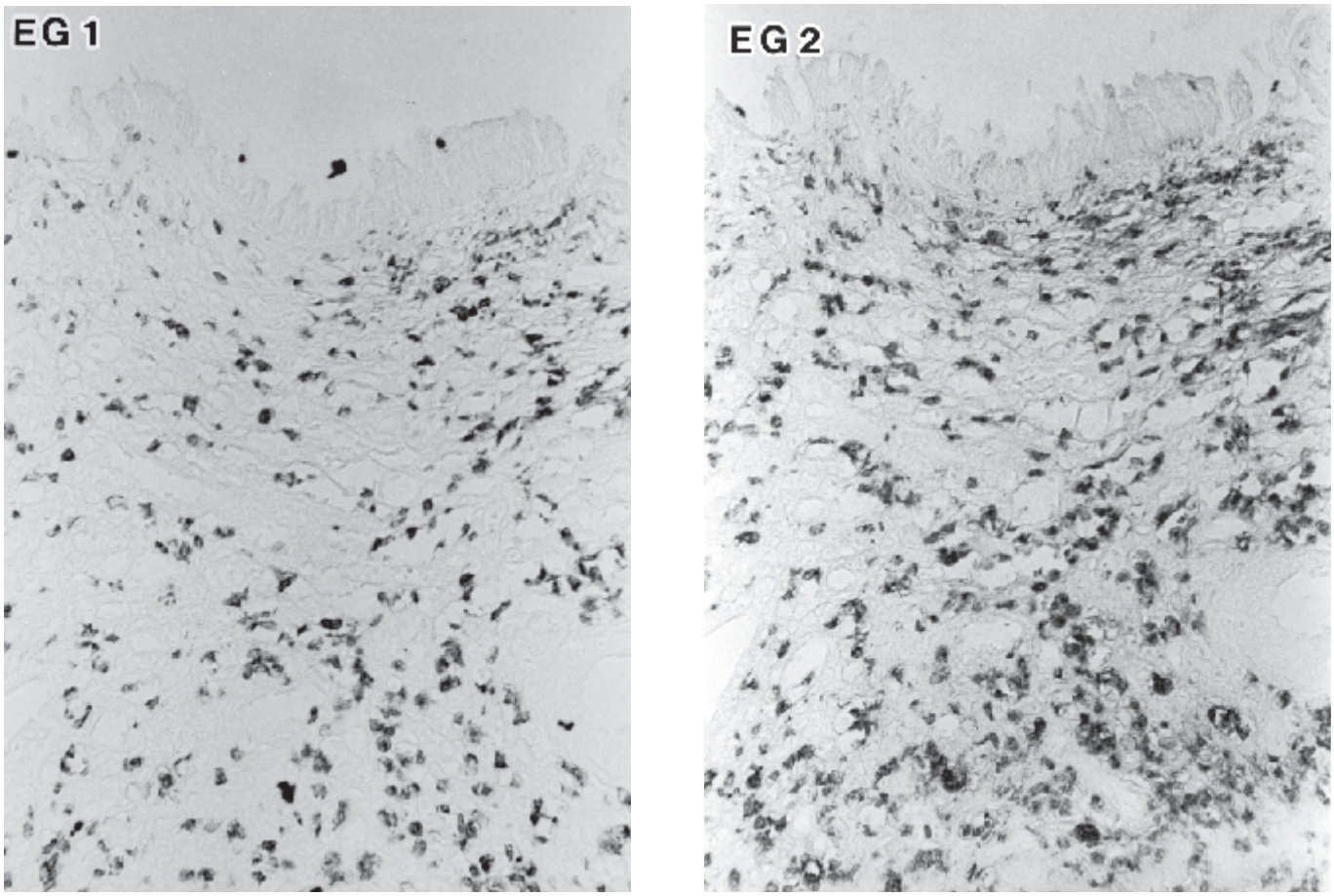

Figure 1. Immunocytochemical labelling of a nasal polyp obtained at surgery (specimen-1). A large number of eosinophils labelled with antibodies to EG1 are visible in the lamina propria. Many activated eosinophils labelled with EG2 antibodies are seen as the EG1-positive eosinophils. × 40.

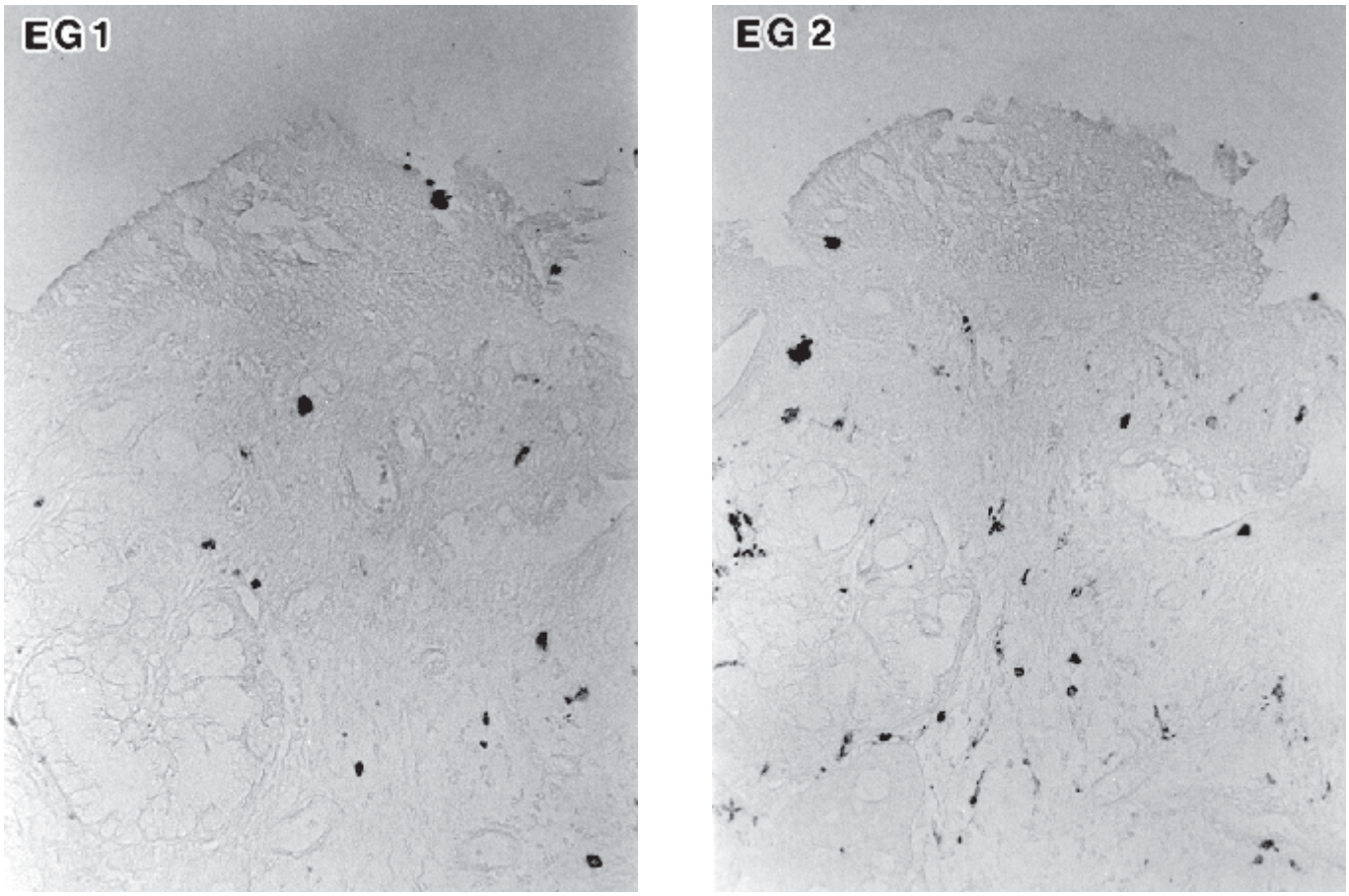

Figure 2. Immunocytochemical labelling of a nasal polyp obtained after steroid treatment (specimen-2). Eosinophils stained with EG1 as well as EG2 are rarely found in any region of the sections. × 40.

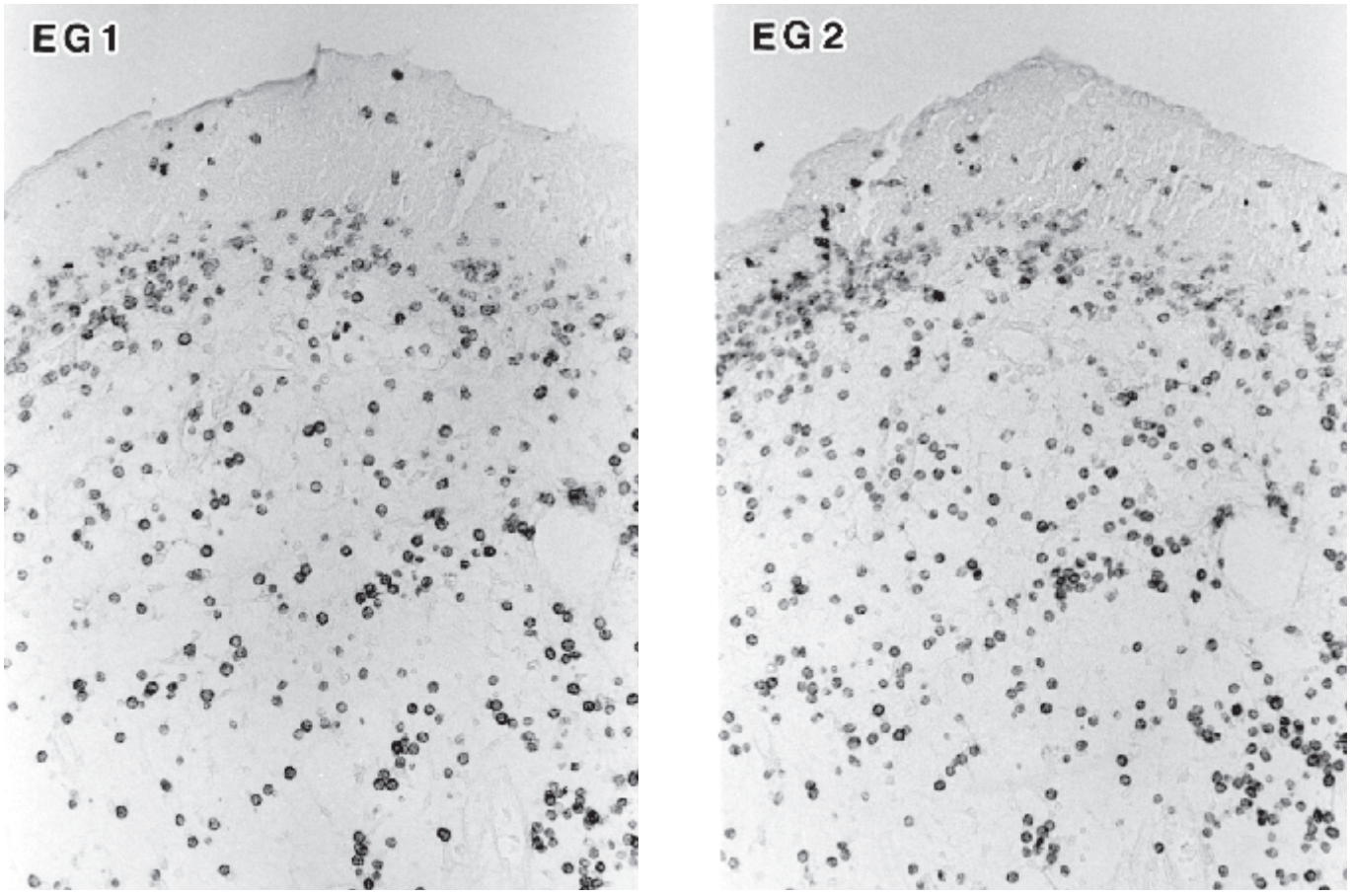

Figure 3. Immunocytochemical labelling of a recurrent nasal polyp (specimen-3). Numerous eosinophils labelled with EG1 antibodies are visible in the lamina propria. Some eosinophils appear to have invaded through the basement membrane into the epithelium. Antibodies to EG2 display the same labelling pattern as the antibodies to EG1. × 40.

tomount ™. A Zeiss Axioskop microscope (equipped with 10 × objective lens) was used for observation and photography. When the primary antibodies to EG1 and EG2 were omitted, no labelling were observed in any sections examined.

Cell counting

The number of positively stained eosinophils was counted in the lamina propria (Igarashi et al., 1993). Ten fields of 100 μm square were examined on the sections of the 3 specimens respectively. The average number of eosinophils per 100 μm^2 and the ratio of EG2-positive to EG1-positive eosinophils were taken as results (Min et al., 1996).

RESULTS

Numerous eosinophils labelled with antibodies to EG1 and EG2 were observed in the lamina propria of the specimen-1 polyps obtained at surgery (Figure 1). In specimen-2 taken immediately after steroid treatment, both EG1- and EG2-positive eosinophils were rarely found in any region of the sections (Figure 2). However, the labelling pattern of eosinophils in the recurrent nasal polyp (specimen-3, Figure 3) paralleled the staining of eosinophils in specimen-1 polyp. A large number of eosinophils labelled with EG1 and EG2 were found, and concentrated infiltration was shown just beneath the epithelium. Some eosinophils appeared to invade through the basement membrane into the epithelium.

The number of EG1-positive eosinophils in specimen-1 was 19.78 ± 2.11/100 μm^2, in specimen-2 2.78 ± 0.64/100 μm^2 and that specimen-3 30.89 ± 3.42/100 μm^2 (Table 1). The number of EG2-positive eosinophils in specimen-1 was 18.67 ± 1.82/100 μm^2, in specimen-2 2.00 ± 0.60/100 μm^2, and in specimen-3 29.78 ± 2.82/100 μm^2 (Table 1). There were significant differences between specimens ($p<0.01$).

The number of both EG1- and EG2-positive eosinophils at recurrence was larger than before treatment. The ratio of EG2-positive to EG1-positive eosinophils in specimen-1 was 0.94, in specimen-2 0.72, and in specimen-3 0.96. This indicates that most of eosinophils infiltrating in the AIA polyps were predominantly composed of the activated and secreted forms of ECP.

Table 1. Mean values (± S.E.) of EG1-positive (+) and EG2+ eosinophils per 100 μm^2 on the sections of nasal polyps obtained at surgery, after steroid treatment and at recurrence, and the ratio of EG2+ to EG1+ eosinophils.

	EG1+	EG2+	EG2+ / EG1+
Specimen-1 (at surgery)	19.78±2.11	18.67±1.82	0.94
Specimen-2 (after steriods)	2.78±0.64	2.00±0.60	0.72
Specimen-3 (at recurrence)	30.89±3.42	29.78±2.82	0.96

DISCUSSION

Accumulation of eosinophils with the other inflammatory cells is frequently observed in nasal polyps (Ogawa, 1986; Jankowski et al., 1989; Jankowski, 1996). A markedly increased number of eosinophils is a primary characteristic of the nasal polyps of AIA patients, suggesting that eosinophil infiltration may be associated with AIA polyp formation (Holopainen et al., 1979; Yamashita et al., 1989; Yoshimi et al., 1993; Jankowski, 1996). The mechanism for local infiltration of the eosinophils in AIA polyps, however, remains to be determined.

Steroids are well known to be effective for the treatment for AIA attacks and also for the management of AIA nasal polyps after surgery (Virolainen and Puhakka, 1980; Karlson and Rundcrantz, 1982). In spite of the clinical and therapeutic features, histological evaluation of eosinophil infiltration in nasal polyps after steroid treatment and at recurrence has not been previously reported. Our immunocytochemical study demonstrated that steroid treatment eliminated nasal polyps and excluded eosinophil accumulation from the polyps, and that re-infiltration of eosinophils was detected at recurrence of polyps. The number of eosinophils at recurrence was larger than the one obtained before treatment. It is interesting that most of the eosinophils accumulated in the AIA polyps were activated before steroid treatment, and the same pattern of eosinophil accumulation was observed when the polyps recurred. The ratio of EG2-positive to EG1-positive eosinophils indicates that most of eosinophils infiltrating in the AIA polyps were predominantly occupied with the activated forms. This ratio is a similar result to that in nasal polyps with atopic patients reported by Min et al. (1996).

The activated eosinophils induce the release of mediators such as major basic protein (MBP) and leukotrienes (LT) from their core granules (Ayars et al., 1985; Probst et al., 1992). Electronmicroscopic demonstration supports the fact that eosinophil degranulation takes place in AIA polyps (Sasaki and Nakahara, 1989). The continued presence of activated eosinophils could start a self-perpetuating process that would remain unaltered without any effective treatment (Moneret-Vautrip et al., 1990; Probst et al., 1992). MBP activates mast cells in which degranulation was found more extensively in the AIA polyp than in allergic rhinitis or chronic sinusitis (Takasaka et al., 1986). Mast cells release chemical mediators such as histamine, prostaglandins, LT, and platelet-activating factor (PAF). PAF is a quite potent chemotactic agent to eosinophils (Furukawa et al., 1992), being 100 times larger than the eosinophil chemotactic factor anaphylaxis (Wardlaw et al., 1986), and also has a potential to increase vascular permeability 1000 times more than histamine (Humphery et al., 1982; Furukawa et al., 1992). These data and our result suggest that activated eosinophils in the AIA polyps might encourage the accumulation of more eosinophils, beginning with the initial stage of polyp formation, by promoting vascular permeability and by the chemotactic factors.

Bascom et al. (1989) demonstrated that steroid treatment significantly reduced the concentration of MBP in the nasal-lavage fluid during the late response to the antigen challenge, and the amount of MBP correlated closely with the number of eosinophils. The major factor accounting for this is, most likely, the

decrease in eosinophil infiltration caused by the steroid treatment (Bascom et al., 1989). Kanai et al. (1994) documented that the proportion of activated eosinophils (EG2 positive) to total eosinophil count was significantly lower in polyps from patients treated with a topical nasal steroid than in polyps from untreated patients. These observations indirectly support our assumption that activated eosinophils may play an important role in accumulation of eosinophils and the formation of nasal polyps in AIA patients.

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