

# Human mast cell progenitors in peripheral blood from atopic subjects with high IgE levels.

I. NOMURA, T. KATSUNUMA, K. MATSUMOTO, M. IIDA, H. TOMITA, M. TOMIKAWA, H. KAWAHARA, A. AKASAWA, R. PAWANKAR\* and H. SAITO

Department of Allergy and Immunology, National Children's Medical Research Center, and \*Department of Otolaryngology, Nippon Medical School, Tokyo, Japan

## Summary

**Background** It remains unclear whether the number of circulating mast cell progenitors is increased in patients with atopic diseases. Distinct genotypes are reported to affect mast cell/basophil activation.

**Objective** We compared the number and function of mast cell progenitors present in the peripheral blood from donors with normal IgE (IgE < 400 U/mL) and those with atopic dermatitis accompanied by high serum IgE (IgE > 5000 U/mL).

**Methods** Purified peripheral blood cells were cultured in serum-free methylcellulose containing stem cell factor (SCF), IL-6 plus IL-3. Fresh methylcellulose containing the cytokines was layered over every 2 weeks. The cultured mast cells were retrieved from the methylcellulose and were functionally analysed.

**Results** Mast cell colonies were distinguished at 6 weeks of culture as other colony types had been degenerated. The number of mast cell colony-forming cells varied depending on donors and was not significantly increased in peripheral blood from the hyper-IgE atopic patients. A significant inversed correlation was found between the number of mast cells per one colony and the ages of donors. The cultured mast cells derived from atopic patients and those from normal IgE donors equally expressed FcεRI and released histamine through FcεRI, although IL-4 priming *in vitro* markedly enhanced the function of mast cells regardless of donors.

**Conclusions** These results indicate that the number of circulating mast cell progenitors may be regulated by unknown individual factors unrelated to IgE levels. Mast cell function may be regulated largely by environmental factors, such as IL-4, but not determined by their progenitors' genotypes.

**Keywords:** FcεRI, mast cells, cell differentiation, IL-4, stem cells

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## Introduction

Mast cells and basophils are the unique cells that can evoke immediate allergic reaction through surface high affinity IgE receptors [1]. Mast cells and basophils develop from distinct progenitors and basophils often share common progenitors with eosinophils [2–4]. Basophils

and eosinophils require IL-3, GM-CSF and IL-5 for their development [5], whereas the development of human mast cells is often independent from IL-3 [6–8] and always dependent on the presence of a factor called a stem cell factor (SCF) or c-kit ligand [9–12].

Mast cells originate from CD34<sup>+</sup> bone marrow progenitors [13,14] and migrate into tissue as an immature form through blood circulation [15,16]. Granulocytes, including basophils, do not migrate from bone marrow while they are developing myelocytes. Although the role of granulocyte

Correspondence: Hirohisa Saito, The Department of Allergy and Immunology, National Children's Medical Research Center, 3–35–31 Taishido, Setagaya-ku, Tokyo 154–8509, Japan. E-mail: hsaito@nch.go.jp

progenitors circulating in peripheral blood is not fully understood, they are more primitive than myelocytes and are released from bone marrow by stresses and stimuli, i.e. exercise, ACTH and endotoxin [17,18]. Cytokines such as G-CSF and GM-CSF [19,20] also can release the primitive haemopoietic progenitors into blood circulation via down-regulation of the integrin  $\alpha 4\beta 1$  [21].

Denburg *et al.* [22] have reported that the number of haemopoietic CD34<sup>+</sup> progenitors having  $\alpha$ -subunit of IL-5 receptor (IL-5R $\alpha$ ) is increased in peripheral blood from atopic subjects, especially while their symptoms are exacerbated. They also have reported [23] that the total number of CD34<sup>+</sup> progenitors is also increased in atopic subjects. Since atopic patients are continuously exposed to many stresses, low levels of allergens and increased cytokines, even in an asymptomatic phase, the increase in the number of lineage-unrelated progenitors may not be specific for pathogenesis of allergic diseases.

In contrast to circulating primitive progenitors giving rise to granulocytes, these progenitors are thought to directly give rise to mast cells in tissue. Thus, it is particularly important to determine whether mast cell progenitors are increased in atopic donors. However, so far, it has not been possible to measure the number of mast cell progenitors in semi-solid culture, since the maturation of mast cells requires an extra-long incubation period compared with granulocytes. We have recently established the long-term methylcellulose culture for human mast cell development [2,24]. Using this technique, we examined the number of mast cell progenitors present in peripheral blood derived from normal volunteers and patients with atopic dermatitis and high IgE levels.

Genotypic polymorphisms, such as high affinity IgE receptor (Fc $\epsilon$ RI)  $\beta$  chain and IL-4/IL-13 signalling [25], are considered to affect atopic diseases, probably through mast cell activation. We also examined the effect of atopic background on mast cell function, such as histamine release, and compared it with the effect of environmental factors, such as addition of IL-4.

## Materials and methods

### Subjects

Seventeen patients with atopic dermatitis, fulfilling the criteria of Hanifin and Rajka, including positive prick testing against house dust mites [26], took part in this study. In order to elucidate the differences between patients and normal volunteers, we selected patients who also had high levels of total IgE (> 5000 U/mL) and anti-*Dermatophagoides farinae* (Df) IgE titres. They had been receiving non-steroid or minimum corticosteroid ointment (dexamethasone valerate and/or hydrocortisone succinate) but not oral corticosteroid

therapy, and were not in exacerbation status. They always had 10–30 points of disease activity scored with the modified Leicester system [27], which was based on five clinical features (erythema, papule, excoriation, oozing and lichenification), graded at six body sites (head, trunk, elbow, hand, knee and foot) on a scale of 0 (none) to 3 (severe), as has been reported [28]. This gave a disease activity score with a maximum of 90 points.

Twelve control subjects whose sera were negative for anti-Df IgE and anti-cedar pollen IgE were chosen for this study. They had low IgE levels (< 400 U/mL) and were negative for a panel of common allergens, such as house dust mites and pollens, by skin prick testing. The volunteers had no atopic symptoms such as seasonal rhinitis. The study was approved by the Ethical Review Board at the National Children's Hospital and all subjects provided their written informed consent prior to entering the study.

### Blood samples

Non-phagocytic mononuclear cells were separated from peripheral blood samples by density-gradient centrifugation using lymphocyte separation medium (Organon Teknica Corp., Durham, NC, USA) after depletion of phagocytes with silica (Immuno Biological Laboratories, Fujioka, Japan). The interface containing mononuclear cells was collected following density-gradient centrifugation. Lineage-negative ( $\text{lin}^-$ ) cells were negatively selected from the mononuclear cells using a magnetic separation column (Macs II, #441-01, Miltenyi Biotec, Bergisch Gladbach, Germany) and a mixture of magnetic microbeads-conjugated antibodies against CD4, CD8, CD11b, CD14 and CD19 (Miltenyi Biotec) according to the manufacturer's instructions. As shown in Table 1, we were able to retrieve  $1.57 (\pm 0.16) \times 10^6 \text{ lin}^-$  cells from  $1.58 (0.16) \times 10^7$  mononuclear cells obtained from 10 mL blood during this procedure (recovery rate was 4.3%–30%).

### Cytokines and antibodies

The following cytokines were added to the cell suspension. Recombinant human (rh) IL-3 was purchased from Intergen, Purchase, NY, USA. rhIL-6 was kindly provided by Kirin Brewery Co. Ltd, Maebashi, Japan. Bulk vials of rhSCF were purchased from PeproTech EC Ltd, London, UK.

### Cell culture

The  $\text{lin}^-$  mononuclear cells obtained from 10 mL blood were suspended in 0.3 mL Iscove's modified Dulbecco's medium (IMDM; GIBCO BRL, Grand Island, NY, USA) supplemented with 1% insulin-transferrin-selenium (GIBCO BRL),  $5 \times 10^{-5} \text{ M}$  2-ME (GIBCO BRL), 1% penicillin +

**Table 1.** Subject characteristics

| Subject | Gender | Age | IgE (U/mL) | Before MACS*       | After MACS†        |
|---------|--------|-----|------------|--------------------|--------------------|
| AD1‡    | M      | 14  | 44556      | $1.03 \times 10^7$ | $1.32 \times 10^6$ |
| AD2     | M      | 24  | 5730       | $0.68 \times 10^7$ | $0.73 \times 10^6$ |
| AD3     | F      | 2   | 57698      | $1.7 \times 10^7$  | $2.03 \times 10^6$ |
| AD4     | M      | 7   | 44678      | $4.1 \times 10^7$  | $3 \times 10^6$    |
| AD5     | M      | 15  | 16944      | $1.4 \times 10^7$  | $0.96 \times 10^6$ |
| AD6     | F      | 6   | 22260      | $3.18 \times 10^7$ | $1.44 \times 10^6$ |
| AD7     | M      | 16  | 6322       | $1.1 \times 10^7$  | $1.07 \times 10^6$ |
| AD8     | F      | 16  | 16396      | $2.5 \times 10^7$  | $2.24 \times 10^6$ |
| AD9     | M      | 17  | 13000      | $1.5 \times 10^7$  | $0.93 \times 10^6$ |
| AD10    | M      | 22  | 20440      | $1.5 \times 10^7$  | $1.21 \times 10^6$ |
| AD11    | M      | 15  | 34819      | $1.8 \times 10^7$  | $0.6 \times 10^6$  |
| AD12    | M      | 36  | 10741      | $1.86 \times 10^7$ | $1.82 \times 10^6$ |
| AD13    | M      | 17  | 16000      | $0.55 \times 10^7$ | $0.92 \times 10^6$ |
| AD14    | M      | 15  | 14810      | $2.14 \times 10^7$ | $1.51 \times 10^6$ |
| AD15    | M      | 15  | 20000      | $1.38 \times 10^7$ | $1.32 \times 10^6$ |
| AD16    | M      | 9   | 12330      | $1.35 \times 10^7$ | $1.92 \times 10^6$ |
| AD17    | M      | 12  | 20885      | $1.15 \times 10^7$ | $1.22 \times 10^6$ |
| NA1§    | M      | 22  | 47.9       | $0.66 \times 10^7$ | $0.53 \times 10^6$ |
| NA2     | F      | 23  | 15.6       | $1.17 \times 10^7$ | $0.78 \times 10^6$ |
| NA3     | F      | 22  | 29.6       | $1.15 \times 10^7$ | $1.23 \times 10^6$ |
| NA4     | M      | 28  | 18.3       | $1.79 \times 10^7$ | $2.34 \times 10^6$ |
| NA5     | F      | 36  | 2.8        | $1.88 \times 10^7$ | $1.41 \times 10^6$ |
| NA6     | F      | 23  | 8.7        | $1.44 \times 10^7$ | $3.36 \times 10^6$ |
| NA7     | F      | 21  | 390        | $1.21 \times 10^7$ | $2.66 \times 10^6$ |
| NA8     | M      | 21  | 145        | $1.39 \times 10^7$ | $2.8 \times 10^6$  |
| NA9     | F      | 24  | 8.7        | Not counted        | Not counted        |
| NA10    | F      | 3   | 25.2       | $1.72 \times 10^7$ | Not counted        |
| NA11    | F      | 8   | 26.9       | Not counted        | Not counted        |
| NA12    | F      | 17  | 5.0        | $0.44 \times 10^7$ | Not counted        |

\*The number of mononuclear cells obtained from 10 mL blood before MACS separation.

†The number of  $\text{lin}^-$  mononuclear cells recovered after MACS separation.

‡Atopic subjects with chronic dermatitis. All of the subjects had positive IgE antibody against house dust mites.

§Non-atopic subjects did not have symptoms of atopic diseases or IgE antibodies against house dust mites and cedar pollen.

streptomycin (GIBCO BRL) and 0.1% BSA (Sigma Co. Ltd, A-7409, St Louis, MO, USA). The cells were mixed well with 2.4 mL serum-free Iscove's methylcellulose medium (Stem Cell Technologies Inc., Vancouver, Canada) and 0.3 mL solutions containing adequate concentrations of SCF, IL-6 and IL-3. The cell suspension was inoculated at 0.3 mL per well in the 24-well plates (Iwaki Glass, Tokyo, Japan) at 37 °C in 5% CO<sub>2</sub>. Every 2 weeks, 0.3 mL of fresh methylcellulose medium containing SCF + IL-6 without cells were layered over the methylcellulose cultures.

### Staining

The differential count of cultured cells was determined based on 100 cells, unless smears had fewer cells, by using cultured

samples centrifuged onto slides with Cytospin 2 (Shandon, Pittsburgh, PA, USA). The cells were examined with May-Grünwald and Giemsa staining or antitryptase immunostaining. Immunostaining for human mast cell tryptase was carried out by using a modified method as previously described [12]. Briefly, the smears were fixed with Carnoy's solution (60% ethanol, 30% chloroform and 10% glacial acetic acid), stained for granular tryptase by the alkaline phosphatase antialkaline phosphatase (APAAP) method using the Dako APAAP Kit (Dako Corporation, Carpinteria, CA, USA) as per the specification of the kit supplier.

### Flow cytometric analysis of *FcεRIα* on mast cells

Mast cells were incubated with saturating concentrations of

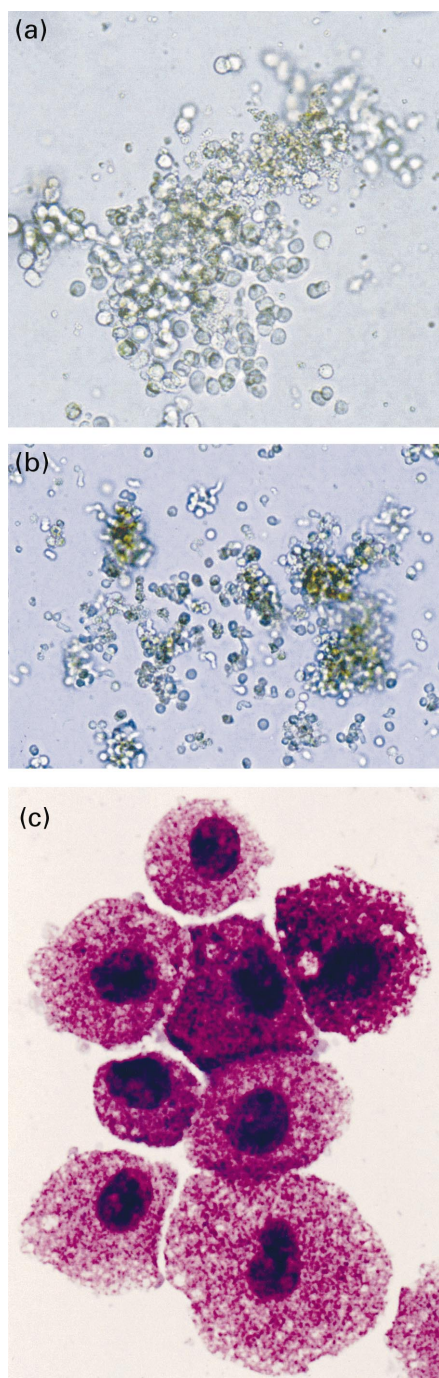


**Fig. 1.** A typical *in situ* morphology of a mast cell colony at 6 weeks of culture. (a) Among large mast cells, degenerated small cells were shown. (b) A typical colony consisting of eosinophils and basophils grown at 3 weeks of culture. Eosinophils tend to form compact subcolonies of small dark cells. (c) May-Grünwald and Giemsa staining of mast cells obtained from a colony. Objective: 10 × (a, b) and 60 × (c).

a mouse IgG<sub>2b</sub> anti-human FcεRIα mAb (CRA-1, Kyokuto Pharmaceutical, Tokyo, Japan) or an identical concentration of irrelevant mouse IgG<sub>2b</sub> (Coulter Immunology, Hialeah, FL, USA) after pretreatment with 50 µg/mL of human IgG (ICN Biomedicals, Aurora, OH, USA). They were then incubated with FITC-conjugated goat anti-mouse IgG (Becton Dickinson, San Jose, CA, USA) for 30 min at 4 °C. After washing twice, the cells were analysed using FACScan (Becton Dickinson, Fullerton, CA, USA) and CellQuest software. Mean fluorescence intensity (MFI) of stained cells was obtained. CRA-1 has been shown to recognize an epitope that is not affected by FcεRI occupancy with IgE [29].

#### Histamine release test

Cells were sensitized with 5 µg/mL human myeloma IgE (a generous gift from Dr Kimishige Ishizaka, La Jolla, CA, USA) with or without addition of IL-4 at 10 ng/mL at 37 °C overnight. After washing, cells were suspended at  $1 \times 10^5$  total cells/mL in modified Tyrode's solution (pH 7.4) containing 124 mM NaCl, 4 mM KCl, 0.64 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM CaCl<sub>2</sub>, 0.6 mM MgCl<sub>2</sub>, 10 mM HEPES and 0.03% HSA. The cells were challenged with either 1.5 µg/mL rabbit anti-human IgE (Dako, Glostrup, Denmark) or control Tyrode's solution at 37 °C for 30 min. Histamine was measured by using an automatic histamine analyser having double HPLC columns (Tosoh, Tokyo, Japan), as reported [12].

#### Statistics

Paired samples were analysed with Wilcoxon matched-pairs signed rank test. Unpaired samples were analysed with Mann-Whitney's *U*-test. When the logarithmic values seemed to be normally distributed, such as colony number or serum IgE levels, regression analysis was done after logarithmic conversion. Statistics were mainly performed on StatView 5.0 for Windows (SAS Institute Inc., Cary, NC, USA). The results were expressed as the mean ± SEM after calculation on Excel 2000 (Microsoft Japan, Tokyo, Japan).

## Results

#### Development of mast cell colonies

We generated mast cell colonies (Fig. 1a) by culturing lin<sup>−</sup> peripheral blood-derived mononuclear cells in serum-free methylcellulose with SCF + IL-6 for 6 weeks. Fresh methylcellulose containing these cytokines was layered over every 2 weeks. IL-3 was added only at the beginning of culture, and it enhanced the mast cell colony growth with

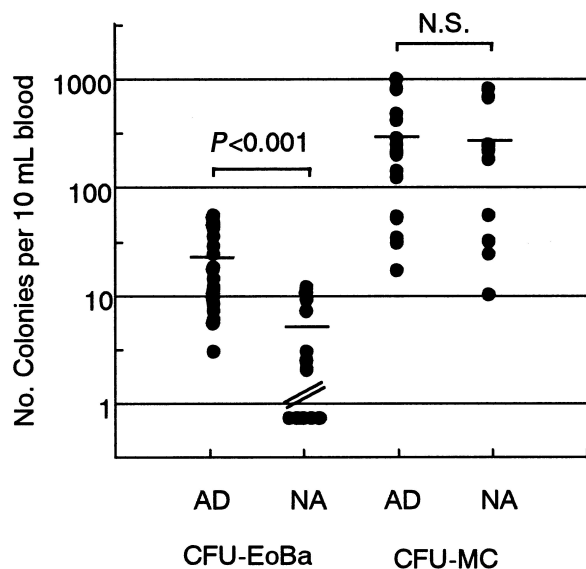


**Fig. 2.** The number of mast cell colonies (6 weeks) and eosinophil/basophil colonies (3 weeks) derived from the patients with atopic dermatitis and normal volunteers. Mast cell colony-forming cells (CFU-MC) and eosinophil/basophil colony-forming cells (CFU-EoBa) were counted *in situ* according to typical appearance (cf. Fig. 1). AD on X-axis means the colonies derived from atopic donors with high IgE titre, and NA means those from non-atopic control donors. CFU-EoBa significantly increased ( $P = 0.0003$ ) in atopic donors, whereas CFU-MC did not ( $P = 0.61$ ).

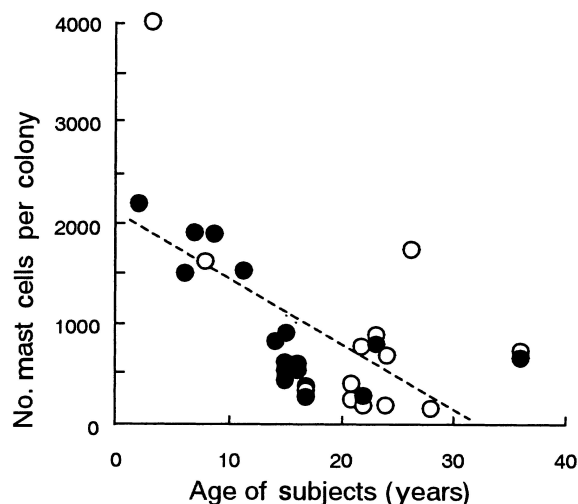


**Fig. 3.** Reverse correlation between the age of subjects and the mast cell number per one colony. Vertical axis represents the number of mast cells per one colony obtained from atopic (closed circles) and non-atopic donors (open circles). Horizontal axis shows the age of donors. The strong inversed correlation was seen ( $r = 0.646$ ,  $P = 0.0002$ ) in both atopic and non-atopic donors. The predicted value was shown as a broken line.

minimum proliferation of macrophage colonies as previously reported [24].

Mast cell colonies were easily detected at 6 weeks of culture because other colony types had been degenerated and >95% of the cells in methylcellulose were always tryptase-positive. At 3 weeks of culture, we were able to detect eosinophil/basophil colonies by typical appearance of *in situ* morphology (Fig. 1b) and by staining of randomly chosen colonies as has been reported [2].

#### Measurement of colony number

Lin<sup>-</sup> mononuclear cells were obtained from normal volunteers and from the patients with atopic dermatitis. As shown in Table 1, all these patients had high IgE titres of >5730 U/mL. The number of mast cell colonies widely varied depending on the samples. The logarithmic values of colony number seem to be normally distributed. The number of mast cell colonies derived from patients' peripheral blood was  $314 \pm 79$  and that from non-atopic volunteers was  $257 \pm 74$ . As shown in Fig. 2, differences between the two logarithmic values were found not to be significant ( $P = 0.61$ ), while those values of eosinophil/basophil colonies were judged to be significantly different ( $25 \pm 5$  vs.  $5 \pm 2$ ,  $P = 0.0003$ ). The mast cell colony number was not correlated with the age (regression:  $r = 0.08$ ,  $P = 0.70$ ) or the serum IgE levels ( $r = 0.03$ ,  $P = 0.90$ ).

At 6 weeks of culture, we dissolved the methylcellulose with PBS and retrieved the cells from all wells. After counting the cell number, cells were stained using May-Grünwald and Giemsa, and antitryptase immunostaining. We found that >95% of cells were mast cells. As shown in Fig. 3, an inversed correlation was found between the average number of mast cells per one colony and the age of donors ( $r = 0.65$ ,  $P = 0.0002$ ). It was difficult to obtain age-matched control subjects as persons who have neither of the IgE antibodies against common allergens are decreasing every year in Japan, especially in the younger generations [30]. Thus, we could recruit only three non-atopic subjects under 20 years of age. Although younger donors tended to have high levels of IgE, eosinophil/basophil colony number and mast cell number per one colony, no significant correlations were found, such as the correlation between the age of donors and the logarithmic number of eosinophil/basophil colonies ( $r = 0.35$ ,  $P = 0.07$ ).

As the number of IL-5 receptor-positive CD34<sup>+</sup> cells has been reported to increase in atopic individuals [22] and the cytokine affects the growth of some mast cells [31,32], we examined the effect of IL-5 on mast cell colony formation. IL-5 at 1 ng/mL was added every 2 weeks with fresh methylcellulose containing SCF + IL-6. The number of

**Table 2.** Comparison of IgE-mediated histamine release and FcεRIα expression on cultured mast cells derived from the patients with atopic dermatitis and normal volunteers

|                        | Atopic subjects |                     |                          | Non-atopic subjects |            |             |
|------------------------|-----------------|---------------------|--------------------------|---------------------|------------|-------------|
|                        | IgE(-)*         | IgE(+) <sup>†</sup> | IgE/IL-4(+) <sup>‡</sup> | IgE(-)              | IgE(+)     | IgE/IL-4(+) |
| Histamine release (%)§ | Not tested      | 14.3 ± 4.4          | 35.6 ± 7.2               | Not tested          | 15.9 ± 2.8 | 38.8 ± 6.3  |
| FcεRIα (MFI ratio)¶    | 1.7 ± 0.1       | 11.7 ± 2.8          | 17.0 ± 2.7               | 1.6 ± 0.3           | 10.6 ± 2.1 | 13.7 ± 0.5  |

\*Cells treated with control medium for 48 h before challenge.

<sup>†</sup>Cells treated with human myeloma IgE at 5 µg/mL for 48 h before challenge.

<sup>‡</sup>Cells treated with human myeloma IgE at 5 µg/mL and IL-4 at 10 ng/mL for 48 h before challenge.

§The cells were challenged with rabbit anti-human IgE antibody 1.5 µg/mL for 30 min. The specific histamine release was obtained by subtracting spontaneous release. The values represent the mean ± SEM of experiments using five–seven subjects.

¶The cells were analysed with flow cytometry after staining with anti-FcεRIα antibody or control antibody. The specific-to-control MFI ratio was indicated as the mean ± SEM of five–seven subjects.

eosinophil/basophil colonies was increased by the addition of IL-5 ( $16.7 \pm 6.0$  vs.  $12.7 \pm 4.5$ ,  $P = 0.007$ ,  $n = 5$ ). However, IL-5 failed to increase the number of mast cell colonies ( $304 \pm 91$  vs.  $308 \pm 93$ ,  $P = 0.74$ ,  $n = 5$ ) or cell number per one colony ( $354 \pm 165$  vs.  $380 \pm 219$ ,  $P = 0.73$ ,  $n = 3$ ).

#### Functional analyses of cultured mast cells

After retrieving the cells from methylcellulose culture at 6 weeks of culture, a part of the cells was further cultured for 4 weeks in liquid medium supplemented with SCF + IL-6. These cells were sensitized with IgE in the presence or absence of IL-4 for 48 h. The cells were then examined for the expression of FcεRIα and anti-IgE-induced release of histamine (Table 2). Again, we failed to observe an increased function of mast cells derived from atopic donors. However, striking differences were found between the values obtained with untreated cells and those obtained with cells treated with IgE and IL-4.

#### Discussion

Human mast cells require an extra-long period for their development from haemopoietic progenitors as compared with other cell types. Therefore, it was very hard to detect mast cell development in semisolid culture, such as in methylcellulose where medium change is not applicable. We were able to generate mast cell colonies in serum-free methylcellulose with SCF + IL-6 for 6 weeks by laying fresh methylcellulose containing these cytokines over the culture every 2 weeks. IL-3 has been reported to be unnecessary for development of cord blood-derived mast cells in serum-free liquid medium under a low oxygen condition [33]. However, the addition of this cytokine at the

beginning of culture was effective for enhancing the mast cell colony development in our study [24].

We failed to find the difference between mast cell progenitor numbers circulating in atopic donors and in non-atopic controls, whereas the number of colonies consisting of eosinophils and basophils was increased in atopic donors as reported [22,23]. It has been reported that IL-5R<sup>+</sup> [22] or CCR3<sup>+</sup> [34] CD34<sup>+</sup> progenitors are increased in peripheral blood during asthma exacerbation. We chose atopic subjects with stable chronic dermatitis in the present study. Although IL-5 did not affect mast cell colony growth in the present study, it would be interesting to examine in a future study whether IL-5R<sup>+</sup> or CCR3<sup>+</sup> CD34<sup>+</sup> progenitors capable of producing of mast cells are increased in acutely exacerbated atopic diseases. Anyway, the number of circulating mast cell progenitors widely varied depending on subjects, suggesting that it is regulated by many hormones and cytokines unrelated to allergic inflammation.

We retrieved the cells at 6 weeks of culture by dissolving methylcellulose culture and used the cells for counting and functional assays. We found the retrieved cells mostly to be mast cells. In our serum-free methylcellulose culture system, macrophages did not develop and eosinophils degenerated after 4 weeks. The average number of mast cells per one colony was inversely correlated with the age of donors. Indeed, we have previously reported that cord blood progenitors give rise to 20-fold more mast cells per one colony compared with adult peripheral blood progenitors when they are cultured in the same condition [35]. Also, the mast cell development from peripheral blood progenitors was hardly detected using donors over 40 years old (data not shown). It is therefore speculated that differentiation capacity of haemopoietic stem cells towards mast cell lineage may be lost earlier in life compared with other haematopoietic lineages. These observations were

consistent with the report that the number of mast cells decreases in tissue after 20 years of age [36]. Mast cell progenitors are abundant in mouse peripheral blood and a few of them are recruited into the tissue where mast cell number is deficient [37]. Therefore, the ability of progenitors to produce mast cells may be more important than the number of mast cell progenitors present in peripheral blood as a regulator of tissue mast cell number.

Kirshenbaum *et al.* have reported [32] the method of generating mast cells from peripheral blood by purifying CD34<sup>+</sup> cells from G-CSF-injected donors. We could generate  $2 \times 10^5$  mast cells from 10 mL peripheral blood without cytokine injection. We employed the negatively selected lin<sup>-</sup> mononuclear cells instead of positive CD34<sup>+</sup> selection, as it was not an efficient way to purify CD34<sup>+</sup> cells from peripheral blood without cytokine injection. From lin<sup>-</sup> mononuclear cells, we observed sufficient mast cell development only by using methylcellulose culture system but not by using liquid culture system (data not shown), suggesting that the contact inhibition by other cell types may be prevented in methylcellulose.

Distinct genotypes have been reported to give different histamine releasability of peripheral blood basophils [38]. Thus, we examined whether the cultured mast cells obtained from atopic donors showed increased histamine releasability or not. Again, we failed to find the increased capacity of the mast cells obtained from atopic donors. On the other hand, treatment of mast cells with IL-4 and IgE significantly enhanced the FcεRI-dependent histamine release. Mast cell function may be regulated largely by environmental factors such as IL-4 but not determined by their progenitors' genotypes.

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