Activated Human Mast Cells Release Factors Supporting Eosinophil Survival in vitro

H.	Hiroshi	Tachimoto ^a
M.	Motohiro	Ebisawa ^{a,b}
Y.	Yoji	Iikura ^{a,c}
M.	Masahiro	Kimata ^a
K.	Kazuhiko	Mori ^a
N.	Naoya	Sakaguchi ^a
A.	Akira	Akasawa ^a
T.	Toshiharu	Nakajima ^a
H.	Hirohisa	Saito ^a

^aDepartment of Allergy, National Children's Medical Research Center, Tokyo,

^bDepartment of Pediatrics, National Sagamihara Hospital, Kanagawa, and ^cDepartment of Pediatrics, Showa University School of Medicine, Tokyo, Japan

Key Words

Mast cells

Eosinophils

GM-CSF

IL-5

Late phase allergic reaction

Correspondence to: Dr. Hirohisa Saito, Department of Allergy, National Children's Medical Research Center, 3-35-31 Taishido, Setagaya-ku, Tokyo (Japan), Tel. +81 3 3414 8121, Fax+81 3 3414 8879

Introduction

Late-phase allergic reaction is characterized by delayed (> 6 h) preferential recruitment of eosinophils at the site of the reaction in response to allergen exposure. Infiltrated eosinophils are thought to survive in the tissue for several days and to change their phenotype into 'hypodense' eosinophils in the presence of eosinophil survival factors. Although mast cells play a crucial role in early-phase allergic reaction, the role of mast cells in late-phase allergic reaction is not well established.

By employing the cultured human mast cells developed in the presence of stem cell factor and interleukin (IL)-6 [1], we have observed that the mast cells sensitized with human IgE produced several cytokines, i.e., tumor necrosis factor (TNF)- α , IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and macrophage inflammatory protein (MIP)-1 α in response to anti-IgE challenge [2].

To further prove the biological cytokine activities in the mast cell supernatants, we investigated the effect of mast cell supernatants on eosinophil survival in vitro. We have found in the present study that eosinophils purified from peripheral blood were mostly able to survive in culture for 3 days in the presence of the supernatant obtained from mast cells activated by anti-IgE challenge but not from nonactivated mast cells. The addition of antibodies against GM-CSF and IL-5 nearly abolished the effect of the supernatant.

from activated mast cells, suggesting that human mast cells are capable of supporting eosinophil survival by producing these cytokines in response to activation through FcεRI.

Materials and Methods

The cultured human mast cells were obtained as reported [1]. Briefly, cord blood mononuclear cells were purified by density centrifugation methods followed by positive selection using monoclonal anti-CD34 antibody in the MACS system to purify CD34⁺ cells. The purified CD34⁺ cells were cultured in medium I (IBL, Men-eki Sei-butsu Kenkyujo, Gunma, Japan) supplemented with 5% fetal calf serum (FCS; Cansera, Rexdale, Canada), 100 ng/ml SCF (Kirin Brewery Co., Ltd., Maebashi, Japan), and 50 ng/ml IL-6 (Kirin) (the mast cell culture medium) for > 10 weeks. The purity of mast cells was determined to be > 99% by May-Grünwald Giemsa (Muto Chemicals, Tokyo, Japan) staining. The cultured mast cells were sensitized with 1 µg/ml human myeloma IgE (a generous gift from Dr. Kimishige Ishizaka) at 37°C overnight, washed, and resuspended at 5×10^3 cells/ml in the mast cell medium. The sensitized mast cells were then challenged with 1.5 µg/ml of rabbit anti-IgE antibody (Dako, Glostrup, Denmark) for 6 h. The supernatants were collected, and then stored at -80°C pending eosinophil survival assays. Human eosinophils were purified by the combination of Percoll (1.090 g/ml) gradient centrifugation and removal of CD 16⁺ neutrophils by using an immunomagnetic beads technique [3]. The purity and viability (trypan blue dye exclusion) of the eosinophils were respectively 96 ± 2 and $98 \pm 1\%$ (mean $\pm$ SEM of 4 experiments). Eosinophil survival assay was done according to a previous report [4]. Eosinophils were resuspended at 1×10^6 cells/ml in RPMI-1640 (IBL) supplemented with 10% FCS (Cansera) and 10% of the mast cell su-

KAKGER

H-Mail karger@karger.ch

Fax+41 61 306 12 34 <http://www.karger.ch>

© 1997 S.KargerAG, Basel

1018 -2438/97/1133- 0293 \$12.00/0

This article is also accessible online at: <http://BioMedNet.com/karger>

pernatants. For negative controls, the mast cell culture medium was added at 10% to the culture. For positive controls, either IL-5 (10 ng/ml, Genzyme, Cambridge, Mass., USA) or GM-CSF (10 ng/ml, R&D System Minneapolis, Minn., USA) was added. The survival of eosinophils was assessed by the trypan blue dye exclusion on days 3 and 6 in culture, and was expressed as the percentage of recovered live cells per originally seeded cells on day 0 in culture. The challenged mast cell supernatants were pretreated with anti-IL-5 antibody (10 µg/ml, R&D System) and anti-GM-CSF antibody (10 µg/ml, R&D System) for 30 min prior to the eosinophil survival assay.

Results and Discussion

The cultured mast cells sensitized with human IgE released > 50% of their histamine content within 10 min [1], and released TNF-α, GM-CSF, IL-5 and MIP-1α at 2ng, 4 ng, 0.1 ng and 4 ng per 10⁶ cells during 6 h incubation [2] in response to the challenge with anti-IgE. As shown in figure 1, eosinophils survived in the presence of activated and nonactivated mast cell supernatants (10⁻⁴ dilution) were respectively 83.5 ± 3.0 and $0.9 \pm 0.4\%$ on day 3 (72 h) in culture. Similar results were obtained on day 6 (144 h) in culture. They were respectively 61.5 ± 5.6 and $0.0 \pm 0.0\%$. Concentration dependency was observed from 10⁻³ to 10⁻⁶ dilution (data not shown). Positive (IL-5 and GM-CSF) and negative controls (the mast cell culture medium) were set to confirm whether these eosinophil survival assays worked appropriately. As shown in

figure 1 , both IL-5 and GM-CSF supported eosinophil survival as has been reported [4], whereas the mast cell culture medium failed to do so. The activity in the supernatants (10⁻⁶ dilution) derived from activated cultured human mast cells for eosinophil survival were almost equivalent to that of IL-5 or GM-CSF at 10 ng/ ml.

The effect of activated mast cell supernatants on eosinophil survival was significantly blocked by the pretreat-ment with anti-IL-5 antibody (26.6 ± 4.4% inhibition) or anti-GM-CSF antibody (52.5±1.7% inhibition). The combi-

Fig. 1. Effect of the supernatants derived from activated or nonactivated mast cells on eosinophil survival. Eosinophils were cultured in the presence of the following factors: medium containing 10% mast cell culture medium (a), rhIL-5 (10 ng/ml) (b), rhGM-CSF (10 ng/ml) (c), the supernatant from 5 × 10⁶ml nonactivated mast cells (10%) (d), the supernatant from 5 × 10⁷ml mast cells activated by anti-IgE challenge (10%) (e), the activated mast cell supernatants neutralized with 10 µg/ml anti-IL-5 antibody (10%) (f), the activated mast cell supernatants neutralized with 10 µg/ml anti-GM-CSF antibody (10%) (g), and the activated mast cell supernatants neutralized with 10 µg/ml anti-IL-5 antibody and 10 µg/ml anti-GM-CSF antibody (10%) (h). The survival of eosinophils was assessed by trypan blue dye exclusion at 72 h of incubation. Each column and a bar represents the mean plus SEM of 4 separate experiments. The differences between e and f, g, h were determined by a double-tailed paired Student t test, and expressed as follows; **p < 0.01, ***p < 0.001.

nation of anti-IL-5 antibody and anti-GM-CSF antibody nearly abolished the eosinophil survival activity (79.7 ± 5.6% inhibition) on day 3 in culture. These results indicate that the mast-cell-derived eosinophil survival activity was mainly due to GM-CSF and IL-5, and that mast cells might play an important role not only in early- but also in late-phase allergic reactions through releasing cytokines active on eosinophils.

References

Saito H, Ebisawa M, Tachimoto H, Shichijo M, Fukagawa K, Matsumoto K, Iikura Y, Awaji T, Tsujimoto G, Yanagida M, Uzumaki H, Taka-hashii G, Tsuji K, Nakahata T: Selective growth of human mast cells induced by Steel factor, IL-6, and prostaglandin E2 from cord blood mononuclear cells. *J Immunol* 1996;157:343-350.

Ebisawa M, Shichijo M, Miura K, Tachimoto H, Katsunuma T, Onda T, Iikura Y, Saito H: Human mast cells produce TNF- α , MIP- α and IL-5 following activation through Fc ϵ R1. *J Allergy Clin Immunol* 1995;95:219.

Ebisawa M, Bochner BS, Georas SN, Schleimer RP: Eosinophil transendothelial migration induced by cytokines. I. Role of endothelial and eosinophil adhesion molecules in IL-1 β -induced transendothelial migration. *J Immunol* 1992; 149:4021-4028.

Fang J, Veksman MY, Ebisawa M, Bochner BS: Increased circulating levels of interleukin-5 in a case of steroid-resistant hypereosinophilic syndrome with dental involvement. *Longcope Forum of the Osier Housestaff. J Allergy Clin Immunol* 1994;94:129-131.

Valerius T, Repp R, Kalden JR, Platzer E: Effects of IFN on human eosinophils in comparison with other cytokines. A novel class of eosinophil activators with delayed onset of action. *J Immunol* 1990;145:2950-2958.

294

Int Arch Allergy Immunol 1997; 113:293-294

Tachimoto/Ebisawa/Iikura/Kimata/Mori/ Sakaguchi/Akasawa/Nakajima/Saito

International Archives of

Allergy^ Immunology