Phenotypic Analysis of Hypodense Eosinophils Derived from Ascites of a Patient with Ascariasis

M.		Ebisawa ^a
M.		Shichijo ^a
H.		Endo ^b
H.		Shimoda ^b
K.		Miura ^a
H.		Saito ^a
Y.		Iikura ^a

^aDivision of Allergy, National Children's Hospital, Tokyo, and ^bDepartment of Pediatrics, Tohoku Rosai Hospital, Sendai, Japan

Key Words

Hypodense eosinophils
Ascariasis
Eosinophilic gastroenteritis
Interleukin-5

Correspondence to: Dr. Motohiro Ebisawa, MD, Division of Allergy, National Children's Hospital 3-35-31, Taishido Setagaya-ku, Tokyo 154 (Japan)

Case Report

A 10-year-old boy was admitted to the Tohoku Rosai hospital due to abdominal pain, vomiting, diarrhea, body weight loss and ascites. The clinical course of the patient is summarized in figure 1. He did not have any history of allergic disease by the time of admission. Stenosis of the pylorus, thickening of gastric mucosa, and ascites were observed by abdominal ultrasonic echo examination. The white blood cell count was 19,300/mm³ with 55% eosinophils. The ascites contained 8,700 white blood cells/mm³ with 99.5% pure eosinophils. Total IgE was 74.9 U/ml, and no specific IgE antibody against foods, mites, or *Ascaris* was detected. Since he was initially diagnosed with eosinophilic gastroenteritis with serosal involvement, he was treated with prednisolone (2 mg/kg/day). A week later after the administration of corticosteroids, eosinophils disappeared completely and clinical symptoms such as diarrhea and ascites were relieved. However, since an *Ascaris* was found in his intestine by radio diagnostic examination a few days later, he further received pyrantel pamoate (300 mg). Two days later, both *Ascaris* eggs and body were found in his stool. We therefore diagnosed this case as eosinophil gastroenteritis with serosal involvement due to ascariasis. Since we were very interested in the selective eosinophil recruitment into ascites in the case, we analyzed density and surface molecules of eosinophils derived from ascites, and mediators including cytokines in ascites.

Methods

Eosinophil density was analyzed using a Percoll (Pharmacia) cen-trifugation method [1]. The amount of interleukin (IL)-5 and other cytokines in serum and ascites was measured by ELISA (R&D Systems). An eosinophil survival assay was performed to evaluate the activation status of eosinophils derived from ascites [2]. Eosinophils were re-suspended in RPMI 1640 (Gibco) with

10% FCS, and cultured in the absence of any cytokines up to 7 days. Viability of eosinophils was determined by trypan blue dye exclusion. Eosinophils derived from ascites were stained with a panel of monoclonal antibodies (5th International Conference on Human Leukocyte Differentiation Antigens), washed, and then stained with FITC-conjugated goat antimouse immunoglobulins [3]. Cells were analyzed using EPICS Profile flow cytometry.

© 1995 S.KargerAG, Basel 1018-2438/95/1073-0346 \$8.00/0

Table 1. Phenotypic analysis of hypodense eosinophils

Cluster
Cluster
Cluster
Cluster
Cluster

(admission)

10/13 10/20 10/27 11/3 11/7

vomiting

... .. diarrhea

Clinical Symptom abdominal pain

‡ aspiration (99.5% Eosinophils, IL-5: 6.3ng/ml)

Prednisolone ‡ : pyrantel pamoate {300mg}

60mq/dav 30 20 10

Eosinophils in
peripheral blood 10.000

($\frac{1}{8} \text{TM}^3/4$ 5,000

IL-5 in serui (pg/ml)

50 -0 -

Fig. 1. Clinical course of patient.

Results of flow cytometric analysis are given in gradings; 1 = < 20%; 2 = 21-40%; 3 = 41-60%; 4 = 61-80%; 5 = > 81%. Comparing with data for normodense eosinophils, expression of CD25, CD54, CDw16 (GM-CSF receptor), and HLA-DR was up-regulated in hypodense eosinophils. In contrast, expression of CD29, CD49d, CD49f, and LeY was down-regulated.

Results and Discussion

The density of eosinophils both in peripheral blood and ascites was almost totally hypodense (< 1.079). IL-5 in serum and ascites was 170 and 6,300 pg/ml, respectively.

Granulocyte/macrophage-colony-stimulating factor (GM-CSF) in ascites was 23.2 pg/ml. IL-3,

IL-4, macrophage inflammatory protein α , and RANTES were not detectable in either ascites or serum. The spontaneous survival of ascites eosinophils was 65% at 48 h, and even 35% at day 7. It is unusual to see survival of normodense eosinophils in vitro without cytokines. Coincubation of ascites eosinophils with dexamethasone (10^{-6} M) reduced the eosinophil survival (0% at day 7). The data suggested that ascites eosinophils had been exposed to IL-5 and GM-CSF in vivo and activated by the cytokines. The results of the surface marker analysis are summarized in table 1. The expression of CD25, CD54, and CDw16 (GM-CSF receptor) was up-regulated compared to the results for normodense eosinophils [3]. These data indicate that eosinophils exposed to IL-5 in ascites became hypodense and activated, and that the hypodense eosinophils expressed CD25, CD54, and CDw16 (GM-CSF receptor) in relation to the activation. Analysis of cytokines and chemokines revealed that the mechanism inducing selective eosinophil recruitment into ascites relied mainly on IL-5 in this case.

References

- 1 Ebisawa M, Bochner BS, Georas SN, Schleimer 2 Fang J, Viksman MY, Eibisawa M, the Longcope 3 Ebisawa M, Schleimer RP, Bickel C, Bochner 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.
- Firm of the Osier Housestuff, Bochner BS: Increased circulating levels of interleukin-5 in a case of steroid-resistant hypereosinophilic syndrome with ileal involvement. *J Allergy Clin Immunol*, in press.
- BS: Phenotyping of purified human peripheral blood eosinophils using blind panel of mAb; in Schlossman SF (ed): *Leukocyte Typing V* Oxford, Oxford University Press, in press.