Characterization of a Receptor for Interleukin 5 on Human Eosinophils: Variable Expression and Induction by Granulocyte/Macrophage Colony-stimulating Factor

By Junichi Chihara, Joël Plumas, Valérie Gruart, Jan Tavernier,* Lionel Prin, André Capron, and Monique Capron

From the Centre d’Immunologie et de Biologie Parasitaire, Unité Mixte INSERM U167, CNRS 624, Institut Pasteur, 59019 Lille, France, and *Roche Research Gent, 9000 Ghent, Belgium

Summary

Interleukin 5 (IL-5) acts on eosinophil differentiation and activation, suggesting the existence of a membrane receptor for IL-5 on eosinophils. Here, we report that 125I-labeled recombinant human IL-5 bound, at 4°C, to high affinity receptors on human eosinophils. The association constant was higher for hypodense eosinophils (1.93 x 10^9 M^-1) than for normodense cells (0.39 x 10^9 M^-1), with a closely related number of receptor sites per cell. No specific binding occurred on neutrophils. The specific binding of 125I was induced by overnight incubation at 37°C of human eosinophils with granulocyte/macrophage (GM)-CSF. The levels of increase were significantly higher for normodense than for hypodense eosinophils, suggesting a previous in vivo activation of the later subpopulation by GM-CSF. IL-3 was ineffective by itself but synergistically enhanced the effect of GM-CSF. Specificity studies showed that the binding of 125I-labeled IL-5 was inhibited by IL-5, but not by other cytokines, on human eosinophils. These results show the existence of a specific binding site for IL-5 on human eosinophils with a variable affinity on eosinophil hypodense or normodense subpopulations, as previously reported for other membrane receptors.

The production and differentiation of eosinophils are thought to be controlled by a group of hemopoietic growth factors, namely, IL-3, granulocyte/macrophage CSF (GM-CSF), and IL-5 (1-3). However, IL-5 appears to be selective for the eosinophil lineage and stimulates the production and maturation of eosinophils at terminal stages (1-3). Human eosinophil heterogeneity is characterized by the existence during hypereosinophilic diseases of a subpopulation of eosinophils called "hypodense" (4-7). Hypodense eosinophils can be distinguished by an impaired oxidative metabolism (6) and by an increased cytotoxic potential against parasitic targets in vitro (7). Moreover, these cells exhibit an increased expression of various membrane receptors (5, 7, 8) besides a variability in protein expression (9). The presence of hypodense eosinophils in hypereosinophilic situations can therefore be related to eosinophils in a state of activation, in addition to increased eosinophilopoiesis. IL-5 might represent the major cytokine involved in the induction of hypodense eosinophils and therefore in eosinophil-mediated pathology (4). Similarly to other lymphokines, the biological effects of IL-5 are probably linked to the interaction with specific receptors on susceptible targets. However, despite numerous studies on IL-5, nothing is known about the binding capacity of IL-5 to human eosinophils. The aim of the present study was to demonstrate the existence of a specific receptor for recombinant human (rh) IL-5 on human eosinophils and to investigate the variation of its expression in relation to eosinophil heterogeneity. The effects of IL-3 and GM-CSF were evaluated on IL-5-specific binding to human eosinophils.

Materials and Methods

Reagents. Purified rh IL-5 was obtained as described elsewhere (10). rh IL-3 and rh GM-CSF were generous gifts from Sandoz, Ltd. (Basel, Switzerland) and Dr. R. L. Coffman (DNAX Institute, Palo Alto, CA), respectively. rh IFN-γ was provided by Roussel-Uclaf (Romainville, France).

Purification of Eosinophils. Human eosinophils were purified from the venous blood of patients with hypereosinophilia by centrifugation upon discontinuous 18–25% (wt/vol) metrizamide gradients (Nyegaard Co., Oslo, Norway) according to the technique previously described (6, 7). Several cell fractions containing >85% pure
eosinophils (range of purity 85–99.7%) were collected from the gradients. Eosinophils obtained in the lower density layers corresponding to 20–22% metrizamide were referred to as hypodense eosinophils. Eosinophils collected from the 23–25% metrizamide layers were referred to as normodense eosinophils (6, 7). Neutrophils purified from normal volunteers by centrifugation on metrizamide gradients were always >95% pure, with no contaminating eosinophils.

**Cell Culture Conditions.** Freshly isolated eosinophils in RPMI 1640 supplemented with 10% FCS and antibiotics were incubated in the presence or absence of rh GM-CSF (5 x 10^{-10} M) and/or rh IL-3 (2 x 10^{-10} M) overnight at 37°C in 5% CO₂. After overnight incubation, eosinophils were used for IL-5 binding assay, in comparison with freshly isolated human eosinophils.

**Radiolabeling of rh IL-5.** rh IL-5 was radiolabeled by the iodogen (Pierce Chemical Co., Rockford, IL) method yielding to a high specific activity with little to no reduction in biological activity, as previously described (11). Briefly, 2 μg of rh IL-5 dissolved in 20 μl PBS was added to a microfuge tube coated with 5 μg of dried iodogen and containing 10 μg of PBS with 500 μCi 125I Na (Amersham International, Amersham, UK). The mixture was incubated at 4°C for 20 min. Radiolabeled rh IL-5 was separated from excess 125I by using a gel filtration column (model PD-10; Pharmacia Fine Chemicals, Piscataway, NJ). The average specific radioactivity was 1.82 ± 0.41 x 10^{-6} cpm/mmol.

**Binding Assay for 125I-labeled rh IL-5.** The binding of 125I-labeled rh IL-5 was measured according to the method described previously (11, 12) with some modifications. Briefly, before addition of various concentrations of 125I-IL-5, the cells (10⁶ in 100 μl HBSS with 0.35% BSA and 0.0025% cycloheximide) were incubated in the presence or absence of a 100-fold excess of unlabeled rh IL-5 for 30 min at 4°C. After incubation, serial dilutions of radiolabeled rh IL-5 were added and the cells were incubated in microfuge tubes at 4°C for 2 h. After washings, the cell suspensions were centrifuged at 8,500 g for 2 min, through 1 ml of 20% sucrose, and the radioactivity of the pellets was measured in a gamma counter (LKB, Turku, Finland). Each experiment was performed in duplicate or more. The specific binding was defined as the difference between the total binding and the nonspecific binding obtained in the presence of a 100-fold molar excess of unlabeled rh IL-5. The association constant (Kₐ) and the average number of binding sites per cell were calculated by Scatchard analysis of the saturation binding data.

**Statistical Analysis.** Data were expressed as mean ± SE. Student’s t test was used for comparison of means, a P value of 0.05 or less being considered significant.

**Results**

**Binding Characteristics of IL-5 on Eosinophils.** A saturation binding experiment with eosinophils using 10⁶ cells per point was performed at 4°C for 2 h in the presence of increasing concentrations of 125I-IL-5. As shown in Fig. 1, 125I-IL-5 specifically bound to human eosinophils and reached saturation at an approximate concentration of 1.0 or 1.25 nM. In this representative experiment, the specific binding of rh IL-5 to normodense and to hypodense eosinophils obtained from the same patient was compared. Scatchard plot analysis of the binding data revealed the presence of 600 receptors per cell with an apparent Kₐ of 1.0 x 10⁹ M⁻¹ on hypodense eosinophils and 490 receptors per cell with a Kₐ of 3.7 x 10⁸ M⁻¹ on normodense eosinophils (Fig. 1, inset). To further investigate the variable expression of IL-5R on human eosinophils, comparative studies of IL-5 binding to hypodense and normodense eosinophils were performed. The results showed that hypodense eosinophils (n = 9) have 446 ± 97 IL-5Rs per cell, with a Kₐ of 1.93 ± 0.4 x 10⁹ M⁻¹, while normodense eosinophils (n = 5) had 460 ± 89 IL-5Rs per cell with a Kₐ of 3.91 ± 0.4 x 10⁹ M⁻¹. The Kₐ of the IL-5R in the case of hypodense eosinophils was fivefold greater than with normodense eosinophils, whereas the number of receptors per cell was not different between the two subpopulations.

The binding of rh IL-5 was evaluated on neutrophils. No significant difference between total and nonspecific binding was observed, indicating that specific IL-5Rs were not detectable on human neutrophils (data not shown).

**Induction of IL-5R on Human Eosinophils.** To further investigate the mechanisms involved in the heterogeneous expression of IL-5R, human eosinophils were cultured overnight in the presence or absence of GM-CSF or IL-3 before the IL-5 binding assay. Results shown in Fig. 2 indicated that IL-5 binding to hypodense eosinophils could be modulated after in vitro incubation with GM-CSF. Whereas eosinophils cultured without GM-CSF expressed a low specific IL-5 binding, the specific IL-5 binding was increased when the same eosinophils had been cultured with GM-CSF. The Scatchard analysis revealed in the later case the existence of 550 receptors per cell with a Kₐ of 2.87 x 10⁹ M⁻¹ (Fig.)
Figure 2. Specific 125I-IL-5 binding to human eosinophils, cultured without GM-CSF (■) or with GM-CSF (■) overnight at 37°C before incubation with increasing concentrations of 125I-IL-5 for 2 h at 4°C. In addition, after culture without GM-CSF, eosinophils were cultured overnight with GM-CSF and submitted to the 125I-IL-5 binding assay (▲). Specific equilibrium binding was determined after subtraction of nonspecific binding and the data were reexpressed as a Scatchard plot analysis (inset). Each point represents the mean of duplicate determinations. In this study, hypodense eosinophils were used.

Results presented in Fig. 3B revealed that IL-3 could not by itself induce the specific binding of IL-5. However, when eosinophils were cultured with GM-CSF and IL-3 together an increase in the specific IL-5 binding to eosinophils was detected, suggesting a synergistic effect between IL-3 and GM-CSF.

Specificity of IL-5 Binding to Human Eosinophils. To determine whether the binding of IL-5 to human eosinophils was specific for IL-5, competition binding experiments with other lymphokines showing amino acid sequence homology with IL-5 in short segments were performed. None of the lymphokines tested—IL-3 (5 × 10^{-6} M), GM-CSF (5 × 10^{-6} M), and IFN-γ (1 × 10^{-6} M)—showed any significant effect on the binding of 125I-IL-5 to human eosinophils (data not shown).

Discussion

IL-5 plays a major role in the production, differentiation, and activation of eosinophils as well as B lymphocytes (12). The pleiotropic effects of IL-5 on hematopoietic cell are probably linked to the expression of specific receptors on susceptible target cells. However, despite the well-known biological effects of IL-5, there is no report on a specific binding site for IL-5 on eosinophils, while a recent study described an IL-5R on a murine B cell line (12). In the present paper,
125I-labeled rh IL-5 was used to demonstrate the existence and to examine the level of expression of a receptor for IL-5 on human eosinophils. The results presented here show that IL-5 specifically interacts on the surface of eosinophils but not on neutrophils, a finding that correlates with the lack of effect of IL-5 on the neutrophil myeloid series. The present results indicate that the binding of IL-5 was to a single class affinity receptor. This is similar to receptors for IL-3 or GM-CSF on human eosinophils (13), but different from the receptor for IL-5 described on the murine B cell line BCL1-B20 (12). From the Scatchard plot analyses, IL-5R expression was different on hypodense and normodense eosinophils. IL-5 bound to hypodense eosinophils with a fivefold higher $K_r$ than for normodense eosinophils, a result that suggests the previous in vivo activation of hypodense eosinophils. In contrast, the number of receptor sites per cell was not different between the two eosinophil cell populations, and was consistent with the small number of high affinity receptors for other lymphokines (IL-3 and GM-CSF) recently described on eosinophils (13). After an overnight incubation of eosinophils at 37°C, in the absence of added cytokines, a specific binding of labeled IL-5 was still detected on hypodense eosinophils but not on normodense populations. These results might indicate that the loss of binding capacity of IL-5 was slower for hypodense than for normodense eosinophils. This is consistent with the fact that hypodense eosinophils have a prolonged life span in vivo and ex vivo without lymphokines (4, 5). Our results on IL-5 binding to hypodense and normodense eosinophils further illustrate the heterogeneity of these two populations already demonstrated for the expression of low-affinity IgE receptors and IgE-mediated cytotoxic function, and the expression of CR3 (7, 8) as well as protein profiles (9). These similarities suggest that the biological changes in hypodense eosinophils might be under the influence of the same factors. In fact, in a different study related to the induction of various surface markers on eosinophils, we could recently show that the expression of CD23, CD11b (CR3), and CD4 was closely related to the effect of eosinophilopoietic growth factors such as IL-5 (Chihara, J., V. Gruart, J. Plumas, A. Capron, and M. Capron, manuscript in preparation).

In the context of the variable expression of IL-5Rs on human eosinophils, it was interesting to investigate the effects of eosinophilopoietic growth factors such as GM-CSF and IL-3 on the specific IL-5 binding. The present results indicate that an overnight culture with GM-CSF could increase the expression of IL-5R not only on hypodense, but also on normodense eosinophils. The level of increase of IL-5 binding after induction by GM-CSF was inferior for hypodense than for normodense eosinophils, suggesting that hypodense eosinophils might have been exposed to an excess of GM-CSF in vivo, and might be deactivated upon further exposure. The enhancing effects of GM-CSF on IL-5 binding to human eosinophils are consistent with the ability of this factor to stimulate eosinophil colonies in vitro. Indeed, eosinophil colonies are selectively induced by IL-5, but further enhanced after the addition of GM-CSF or IL-3 (1, 3). In fact, IL-3 alone could not induce IL-5 binding to human eosinophils. However, in synergy with GM-CSF, IL-3 could increase IL-5 binding to human eosinophils. These findings are not in total agreement with a recent study showing a reciprocal inhibition of binding to human eosinophils between IL-3 and GM-CSF and suggesting common biological effects (13). In our studies, the binding or radiolabeled IL-5 to eosinophils was found to be inhibited only by unlabeled IL-5 and not by IL-3, GM-CSF, IFN-γ suggesting the specificity of the binding site for IL-5 on human eosinophils. The characteristics of IL-5 binding to human eosinophils and to murine B cells are not totally identical, especially with respect to the presence of a single class affinity receptor for eosinophils, whereas high- and low-affinity receptors are detected on B cell lines (12). Furthermore, mAbs to mouse B cell IL-5Rs did not bind to human eosinophils (data not shown). Because of the limited amount of eosinophils from human patients, only preliminary cross-linking studies have been performed. They suggest that the IL-5R on human eosinophils has an approximate molecular weight of 50,000. The molecular relationships between the IL-5R on eosinophils and on B lymphocytes await further investigation.

The authors gratefully acknowledge Mr. K. Rüedi (Sandoz, Ltd.), and Dr. R. L. Coffman (DNAX) for their generous supply of essential reagents for this study.

This work was supported by Unité Mixte INSERM U 167-CNRS 624. Junichi Chihara was the recipient of a postdoctoral fellowship of INSERM and Fondation pour la Recherche Médicale, France.

Address correspondence to Monique Capron, Centre d'Immunologie et de Biologie Parasitaire, Institut Pasteur, B.P. 245, 59019 Lille, Cédex, France. J. Chihara's present address is 4th Department of Internal Medicine, Kinki University, School of Medicine, Osaka, Japan.

Received for publication 28 March 1990 and in revised form 13 August 1990.

Characterization of a Receptor for Interleukin 5 on Human Eosinophils
References

1. Sonoda, Y., N. Arai, and M. Ogawa. 1989. Human regulation of eosinophilopoiesis in vitro: analysis of the targets of interleukin-3, granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-5. *Leukemia (Baltimore)*. 3:14.

2. Saito, H., K. Hatake, A.M. Dvorak, K.M. Leiferman, A.D. Donnenberg, N. Arai, K. Ishizaka, and T. Ishizaka. 1988. Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. *Proc. Natl. Acad. Sci. USA*. 85:2288.

3. Clutterbuck, E.J., E.M.A. Hirst, and C.J. Sanderson. 1989. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. *Blood*. 73:1504.

4. Owen, W.F., M.E. Rothenberg, J. Peterson, P.F. Weller, D. Silberstein, A.L. Sheffer, R.L. Stevens, R.J. Soberman, and K.F. Austen. 1989. Interleukin-5 and phenotypically altered eosinophils in the blood of patients with the idiopathic hypereosinophilic syndrome. *J. Exp. Med.* 170:343.

5. Chihara, J., and S. Nakajima. 1989. Induction of hypodense eosinophils and nuclear hypersegmentation of eosinophils by various chemotactic factors and lymphokines in vitro. *Allergy Proceedings*. 10:27.

6. Prin, L., J. Charon, M. Capron, P. Gosset, H. Taelman, A.B. Tunnel, and A. Capron. 1984. Heterogeneity of human eosinophils. II. Variability of respiratory burst activity related to cell density. *Clin. Exp. Immunol.* 57:735.

7. Capron, M., H.L. Spiegelberg, L. Prin, H. Bennich, A.E. Butterworth, R.J. Pierce, M.A. Ouaissi, and A. Capron. 1984. Role of IgE receptors in effector function of human eosinophils. *J. Immunol.* 132:462.

8. Capron, M., M.D. Kazatchkine, E. Fischer, M. Joseph, A.E. Butterworth, J.P. Kusnierz, L. Prin, J.P. Papin, and A. Capron. 1987. Functional role of the alpha-chain of complement receptor type 3 in human eosinophil-dependent antibody-mediated cytotoxicity against schistosomes. *J. Immunol.* 139:2059.

9. Gruart, V., J.M. Balloul, L. Prin, M. Tomassini, S. Loiseau, A. Capron, and M. Capron. 1989. Variations in protein expression related to human eosinophil heterogeneity. *J. Immunol.* 142:4416.

10. Tavernier, J., R. Devos, J. Van der Heyden, G. Hauquier, R. Bauden, I. Fache, E. Kawai, J. Vandekerckhove, R. Contras, and W. Fiers. 1989. Expression of human and murine interleukin-5 in eukaryotic systems. *DNA (NY)*. 8:491.

11. Lowenthal, J.W., B.E. Castle, J. Christiansen, J. Schreurs, D. Rennick, N. Arai, P. Hoy, Y. Takebe, and M. Howard. 1988. Expression of high affinity receptors for murine interleukin-4 (BSF-1) on hemopoietic and non-hemopoietic cells. *J. Immunol.* 140:456.

12. Mita, S., N. Harada, S. Naomi, Y. Hitoshi, K. Sakamoto, M. Akagi, A. Tominaga, and K. Takatsu. 1988. Receptors for T cell-replacing factor/interleukin 5: specificity, quantitation, and its implication. *J. Exp. Med.* 168:863.

13. Lopez, A.F., J.M. Eglinton, D. Gillis, L.S. Park, S. Clark, and M.A. Vadas. 1989. Reciprocal inhibition of binding between interleukin-3 and granulocyte-macrophage colony-stimulating factor to human eosinophils. *Proc. Natl. Acad. Sci. USA*. 86:7022.