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

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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 ^{125}I-labeled recombinant human IL-5 bound, at 4°C, to high affinity receptors on human eosinophils. The association constant was higher for hypodense eosinophils ($1.93 \times 10^9\ M^{-1}$) than for normodense cells ($0.39 \times 10^9\ M^{-1}$), with a closely related number of receptor sites per cell. No specific binding occurred on neutrophils. The specific binding of IL-5 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 ^{125}I-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),[1] 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.

[1] *Abbreviations used in this paper:* CR3, complement receptor type 3; GM-CSF, granulocyte/macrophage colony stimulating factor; K_a, association constant; rh, recombinant human.

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

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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 (5×10^{-10} M) and/or rh IL-3 (2×10^{-10} M) overnight at 37°C in 5% CO_2. 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 ^{125}I-Na (Amersham International, Amersham, UK). The mixture was incubated at 4°C for 20 min. Radiolabeled rh IL-5 was separated from excess ^{125}I by using a gel filtration column (model PD-10; Pharmacia Fine Chemicals, Piscataway, NJ). The average specific radioactivity was $1.82 \pm 0.41 \times 10^{15}$ cpm/mmol.

Binding Assay for ^{125}I-labeled rh IL-5. The binding of ^{125}I-labeled rh IL-5 was measured according to the method described previously (11, 12) with some modifications. Briefly, before addition of various concentrations of ^{125}I-IL-5, the cells (10^6 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 (K_a) 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 10^6 cells per point was performed at 4°C for 2 h in the presence of increasing concentrations of ^{125}I-IL-5. As shown in Fig. 1, ^{125}I-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 IL5 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 K_a of 1.0×10^9 M^{-1} on hypodense eosinophils and 490 receptors per cell with a K_a of 3.7×10^8 M^{-1} on normodense eosinophils (Fig. 1, *inset*). To

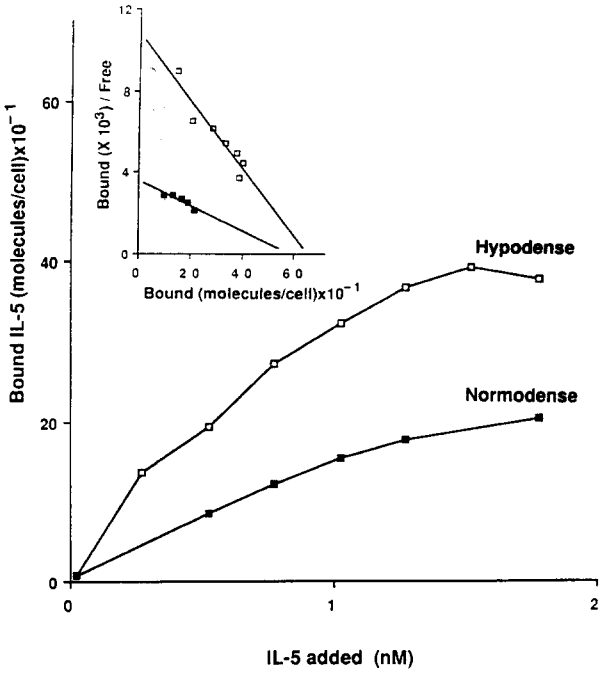

Figure 1. Specific equilibrium binding curve of ^{125}I-IL-5 to human eosinophils. Hypodense eosinophils (□) and normodense eosinophils (■), obtained from the same donor, were incubated with various concentrations of ^{125}I-IL-5 for 2 h at 4°C. The specific binding was determined after subtraction of nonspecific binding and the data were reexpressed as a Scatchard plot analysis (*inset*). Each point represents the mean of duplicate determinations.

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 cells, with a K_a of $1.93 \pm 0.4 \times 10^9$ M^{-1}, while normodense eosinophils ($n = 5$) had 460 ± 89 IL-5Rs per cell with a K_a of $3.91 \pm 0.43 \times 10^8$ M^{-1}. The K_a 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 IL5 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 K_a of 2.87×10^9 M^{-1} (Fig.

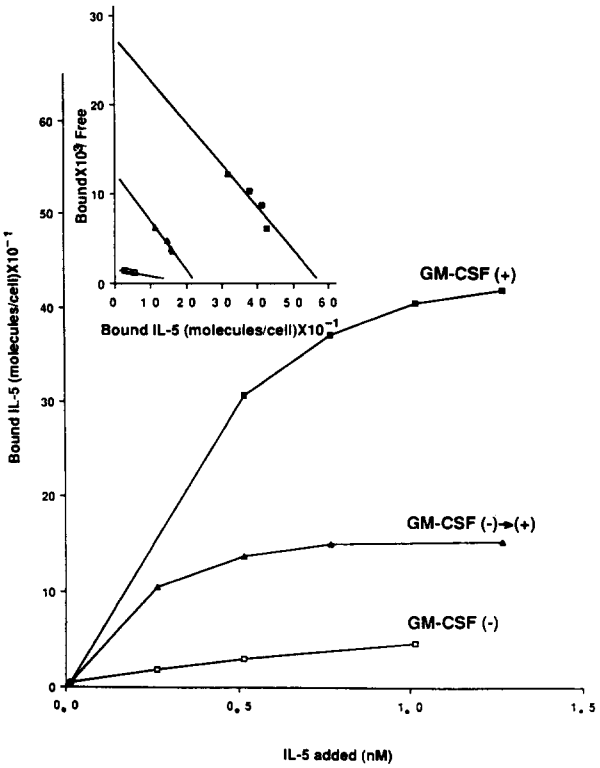

Figure 2. Specific ^{125}I-IL-5 binding to human eosinophils, cultured without GM-CSF (□) or with GM-CSF (■) overnight at 37°C before incubation with increasing concentrations of ^{125}I-IL-5 for 2 h at 4°C. In addition, after culture without GM-CSF, eosinophils were cultured overnight with GM-CSF and submitted to the ^{125}I-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.

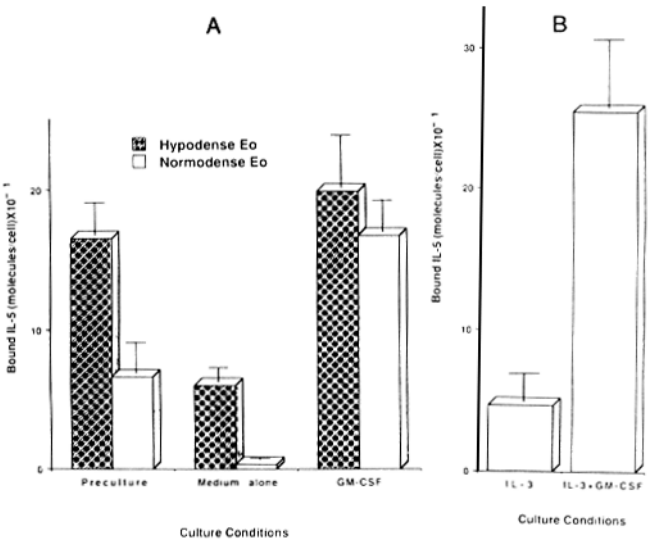

Figure 3. (*A*) Effect of GM-CSF on specific IL-5 binding to human eosinophils. Human eosinophils, freshly isolated (preculture), or cultured overnight without GM-CSF (medium alone) or with GM-CSF, were incubated with 0.5 nM of ^{125}I-IL-5 for 2 h at 4°C. Specific IL-5 binding to hypodense eosinophils (▨, n = 9) or normodense eosinophils (□, n = 5) was determined after subtraction of nonspecific binding. Data are expressed as mean ± SE. (*B*) The effect of IL-3 alone, or IL-3 + GM-CSF on specific IL-5 binding to human eosinophils (four experiments with hypodense eosinophils and one experiment with normodense eosinophils). Human eosinophils, cultured with IL-3, or with IL-3 + GM-CSF, were incubated with the same concentration of ^{125}I-IL-5 (0.5 nM) for 2 h at 4°C. Data are expressed as mean ± SE of five independent experiments.

2, *inset*). In addition, when human eosinophils first cultured without GM-CSF were then cultured with GM-CSF overnight, a specific binding of IL-5 was now expressed. The Scatchard analysis revealed the existence of 210 receptors per cell with a K_a of 3.2×10^9 M^{-1} (Fig. 2, *inset*). In the same conditions, human eosinophils cultured with IL-3 did not specifically bind IL-5 similarly to eosinophils cultured without lymphokines (data not shown). To extend these findings, the specific IL-5 binding was evaluated on normodense and hypodense eosinophils cultured overnight in the presence or absence of GM-CSF before incubation with ^{125}I-IL-5 (Fig. 3 *A*). As previously shown, in the case of freshly isolated eosinophils (preculture conditions), the specific IL-5 binding was greater on hypodense than on normodense eosinophils. After culture without GM-CSF (medium alone), the specific IL-5 binding was still detected on hypodense eosinophils, but not on normodense eosinophils. However, cultivation with GM-CSF induced IL-5–specific binding both on hypodense or normodense eosinophils. It can be also noticed on Fig. 3 *A* that the increasing effects of GM-CSF, when compared with precultured eosinophils, were significantly higher in the case of normodense than in the case of hypodense eosinophils.

Results presented in Fig. 3 *B* 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 ^{125}I-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 cells 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,

^{125}I-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_a 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.

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