Interleukin-5, Interleukin-3, and Granulocyte-Macrophage Colony-stimulating Factor Cross-compete for Binding to Cell Surface Receptors on Human Eosinophils*

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Human interleukin (IL)-5 receptors were characterized by means of binding studies using bioactive 125I-labeled IL-5. Of purified primary myeloid cells, eosinophils and basophils but not neutrophils or monocytes expressed surface receptors for IL-5. Binding studies showed that eosinophils expressed a single class of high affinity receptors (Kd = 1.2 x 10^-9 M^-1) with the number of receptors being small (<1000 receptors/cell) and varying between individuals. Among several cell lines examined only HL-60 cells showed detectable IL-5 receptors which were small in numbers (200 receptors/cell) and also bound 125I-IL-5 with high affinity. The binding of IL-5 was rapid at 37 °C while requiring several hours to reach equilibrium at 4 °C. Specificity studies revealed that the two other human eosinophilopoietic cytokines IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) inhibited the binding of 125I-IL-5 to eosinophils. No competition was observed by other eosinophil activating or nonactivating cytokines.

The inhibition of 125I-IL-5 binding by IL-3 and GM-CSF was partial up to a concentration of 10^-7 M with GM-CSF consistently being the stronger competitor. Converse experiments using IL-5 as a competitor revealed that this cytokine inhibited the binding of 125I-IL-3 and of 125I-GM-CSF in some but not all the individuals tested, perhaps reflecting eosinophil heterogeneity in vivo. Cross-linking experiments on HL-60 cells demonstrated two IL-5-containing complexes of M, 150,000 and M, 80,000 both of which were inhibited by GM-CSF.

The competition between IL-5, IL-3, and GM-CSF on the surface of mature eosinophils may represent a unifying mechanism that may help explain the common biological effects of these three eosinophilopoietic cytokines on eosinophil function. This unique pattern of competition may also be beneficial to the host by preventing excessive eosinophil stimulation.

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The abbreviations used are: IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; h, human; m, mouse; PAGF, polyacrylamide gel electrophoresis; r, recombinant; SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Human interleukin-5 (IL-5) is a T cell-derived cytokine responsible for the production of eosinophils from bone marrow cells and for their survival and functional activation in vitro (1-4). Recent studies on patients with hypereosinophilic syndrome and the eosinophilia-myalgia syndrome suggest that human IL-5 also exhibits these properties in vivo (5, 6).

Although stimulating preferentially the eosinophil lineage, IL-5 has been so far shown to stimulate the same pattern of functional responses as IL-3 and GM-CSF. For example, mouse (m) and human (h) IL-5, like hIL-3 and hGM-CSF, enhance h-eosinophil antibody-dependent killing of tumor cells (2, 7-9) and hemihepatins (10-12), stimulate phagocytosis of yeast (2, 7-9), increase the expression of the complement receptor type 3 (7, 8), prolong eosinophil survival (10-13), increase the biosynthesis of proteoglycans (14), and facilitate the conversion of normodense eosinophils to the hypodense phenotype (10-12). Importantly, not only the spectrum of functions but also the magnitude of the stimulation of each eosinophil function by IL-5, IL-3, and GM-CSF is very similar. These results raised the possibility that all three eosinophil growth factors have a common step in their signaling mechanism. In addition, the cross-reactivity of mIL-5 with human eosinophils suggested that this cytokine recognised the human IL-5 receptor.

We have investigated the distribution of the human IL-5 receptor on several primary cell types and cell lines, the kinetics of IL-5 binding, and the molecular species involved in binding to IL-5. In particular we examined the relationship between human eosinophil IL-5 receptors and those for IL-3 and GM-CSF. Significantly the eosinophil IL-5 receptor interacted with IL-3 and GM-CSF but not with other cytokines. It is proposed that this interaction is an important determinant in unifying signaling on human eosinophils and in preventing their excessive stimulation in vivo.

**EXPERIMENTAL PROCEDURES**

**Purification of Human Eosinophils and Other Myeloid Cells—Eosinophils were obtained from the peripheral blood of eosinophilic (9-34%) individuals or from pleural effusions (60-85% eosinophils) and purified by centrifugation on a hypertonite gradient of metrizamide as described (15). The eosinophil preparations were always >93% pure.**

Highly enriched human basophils were obtained from a patient with end-stage Philadelphia chromosome-positive chronic granulomatous leukemia undergoing basophilic differentiation. After dextran sedimentation, basophils were purified by centrifugation on a hypertonite gradient of metrizamide. The 20% metrizamide fractions containing >90% basophils, as judged by Alcian Blue staining, were used for binding studies. Neutrophils were purified from normal volunteers by centrifugation on a hypertonite gradient of metrizamide as described (15). Monocytes were purified by countercurrent elutriation. Mononuclear cells were washed twice in RPMI 1640 at 150 x g and resuspended in medium containing 0.1% heat-inactivated human AB serum. The cells were separated in a Beckman J-GM/E elutriator.
Cross-competing of Eosinophil Growth Factors

(Palo Alto, CA) using the Sanderson chamber, with a rotor speed of 2050 rpm and a flow rate of 11.8 ml/min. Cells remaining in the chamber after 30 min were collected, washed twice in RPMI, and used immediately. This method resulted in a monocyte purity by Wright’s-Giemsa staining of 91% with >90% of elutriated cells being Wright’s-Giemsa stained immediately. This method resulted in high specific activity (17, 18).

Cross-linking and SDS-Polyacrylamide Gel Electrophoresis (PAGE)—HL-60 cells were incubated with 125I-IL-5 (1 × 10^6 m) in the presence of unlabeled homologous and heterologous ligands for 1 h at 4 °C. After incubation, the cells were processed as above.

Association experiments and curve fitting were carried out as described elsewhere.

Cross-linking and SDS-Polyacrylamide Gel Electrophoresis (PAGE)—HL-60 cells were incubated with [125I]-IL-5 (1 × 10^6 m) in the presence or absence of unlabeled IL-5 at 10^6 m. After incubation, the cells were processed as above.

Association experiments and curve fitting were carried out as described elsewhere.

Characterization of [125I]-Labeled IL-5—Purified human IL-5 was radiolabeled to high specific activity (2-6 × 10^6 cpm/pmol) using the iodine monochloride method, the Enzyme-conjugated radiolabeled reagent (Bio-Rad), or the Bolton-Hunter reagent as described under “Experimental Procedures.” SDS-PAGE analysis of [125I]-IL-5 under nonreducing and reducing conditions showed that the native molecule was a dimer (Fig. 1A).

A low molecular weight band was also seen probably representing free iodine at the dye front. Functional testing of [125I]-IL-5 labeled by the iodine monochloride method in an eosinophil polarization assay (7) showed little or no loss of biological activity (Fig. 1B). The other two labeling methods produced similar retention of biological activity (data not shown).

Characterization of [125I]-IL-5 Binding—The kinetics of [125I]-IL-5 binding at different temperatures was established using a clone of the myeloid cell line HL-60 which had been selected for higher expression of IL-5 receptors. Fig. 2A shows binding of [125I]-IL-5 to HL-60 cells at 4, 25, and 37 °C. At 25 and 37 °C the binding was rapid and saturable, reaching apparent equilibrium by 1-2 h. Binding at 4 °C was much slower, requiring >3 h to reach equilibrium. Fig. 2B illustrates the association kinetics at 37 °C of [125I]-IL-5 binding to HL-60 cells, over a broader concentration range. Based on these data, 60 min was selected as the optimal time period for binding experiments at 37 °C to both allow achievement of stable maximum binding while minimizing the length of incubation. Sodium azide is included in all incubations at 37 °C to minimize internalization of ligand, however, at least with the myeloid cell line HL-60, some internalization does occur over this time period. In this case, the conditions for true binding equilibrium have not been met and the binding parameters obtained represent values for the case in which IL-5 binding has reached an experimentally determined stable maximum.

Fig. 3 illustrates typical equilibrium binding data for [125I]-

Fig. 1. Characterization of [125I]-IL-5 by SDS-PAGE and eosinophil polarization assay. Panel A, autoradiograph of [125I]-labeled IL-5 boiled in the presence (reduced) or absence (nonreduced) of 2-mercaptoethanol and applied onto a 10% polyacrylamide gel (panel B). Percent eosinophils polarized in the presence of different concentrations of unlabeled (●) and [125I]-labeled IL-5 (●). Each value is the mean of triplicate determinations.
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± 1.7 × 10^9 M⁻¹ with 180 ± 70 specific binding sites/cell (average from 14 binding experiments) using IL-5 radiolabeled with Bolton-Hunter reagent, and 6.6 ± 3.0 × 10^9 M⁻¹ with 200 ± 90 specific binding sites/cell (average from four binding experiments) using 125I-IL-5 radiolabeled with the Enzybate reagent. These results verify that IL-5 radiolabeled on either tyrosine or lysine residues exhibits essentially identical binding characteristics.

**Cellular Distribution of the IL-5 Receptor**—Human eosinophils and basophils, but not neutrophils, monocytes, or lymphocytes purified from peripheral blood, bound 125I-IL-5. (Table I). Tissue eosinophils obtained from two patients with pleural effusions also expressed IL-5 receptors although these were much fewer in numbers than blood eosinophils (Table I). Basophils from a patient with chronic myeloid leukemia undergoing basophilic differentiation expressed similar numbers of receptors to eosinophils. Examination of several cell lines under high affinity conditions demonstrated that only the myeloid cell line HL-60 expressed IL-5 receptors while in all other myeloid cell lines tested IL-5 receptors were undetected (Table I). Non-myeloid cell lines including cells of T cell, B cell, and pre-B cell origin also lacked high affinity IL-5 receptors.

**Specificity of IL-5 Binding**—In order to ascertain the specificity of 125I-IL-5 binding, different cytokines at 100-fold or greater molar excess concentration were incubated with either eosinophils or HL-60 cells and a subsaturating concentration of 125I-IL-5. The results showed that not only IL-5 but also GM-CSF and IL-3 inhibited the binding of 125I-IL-5 to eosinophils (Table II). Similarly, GM-CSF was also able to inhibit the binding of 125I-IL-5 to HL-60 cells, while IL-3, which does not bind to HL-60 cells, was ineffective. In contrast, a number of other cytokines did not inhibit binding to either cell type. Mouse IL-5, like human IL-5, fully competed for 125I-IL-5 binding on eosinophils.

**Table I**

### Cellular distribution of human IL-5 receptors

| Cell lines | Phenotype | High affinity receptors/10^6 cells |
|------------|-----------|----------------------------------|
| HL-60      | Promyelocytic leukemia | 200                             |
| U937       | Promonocytic leukemia   | <20                             |
| KG1        | Myelogenous leukemia     | <20                             |
| K562       | Erythroleukemia          | <20                             |
| RPMI 8401  | T lymphoma               | <20                             |
| JURKAT     | T lymphocyte             | <20                             |
| ARH77      | B lymphoma               | <20                             |
| RPMI 1788  | B lymphoma               | <20                             |
| RAJ1       | B lymphoma               | <20                             |
| CESS       | B lymphoma               | <20                             |
| Nalm 6     | Pre-B lymphoma           | <20                             |
| CRL 8083   | Myeloma                  | <20                             |

Fig. 2. Kinetics of 125I-IL-5 binding. HL-60 cells (3.3 × 10^7 cells/ml) were incubated with 4 × 10^-10 M 125I-IL-5 at 4 °C (O), 25 °C (■), and 37 °C (△) (panel A) or with 9.6 × 10^-13 M (A), 3.5 × 10^-12 M (B), or 2 × 10^-11 M (O) 125I-IL-5 at 37 °C (panel B). At the indicated times aliquotes were removed and assayed for binding as previously described (33). Data are corrected for nonspecific binding measured in the presence of excess unlabeled IL-5.

Fig. 3. Equilibrium binding and Scatchard analysis (insets) of 125I-IL-5 binding to human eosinophils (panel A) and HL-60 cells (panel B). 2 × 10^5 eosinophils (85% pure) were incubated with various concentrations of 125I-IL-5 (specific activity 4.8 × 10^6 cpm/pmol) for 18 h at 4 °C. 5 × 10^5 HL-60 cells were incubated with various concentrations of 125I-IL-5 (specific activity 4.6 × 10^6 cpm/pmol) for 60 min at 37 °C. Data are corrected for nonspecific binding measured in the presence of >100-fold molar excess of unlabeled IL-5.

IL-5 to eosinophils at 4 °C (panel A) and binding to HL-60 cells at 37 °C in vitro under the conditions described above (panel B). In both cases Scatchard analysis of the data yielded a straight line, indicating a single class of high affinity binding sites for IL-5 on both cell types. The data in Fig. 3A show that eosinophils bound to 125I-IL-5 with a calculated Kd of 1.2 × 10^-9 M⁻¹ and expressed 218 specific binding sites/cell. Similar results were obtained in two other experiments (data not shown). For HL-60 cells, the apparent calculated Kd was 6.2
TABLE II
Specificity of the human IL-5 receptor

| Competitor | % inhibition of 125I-IL-5 binding to | Eosinophils | HL-60 cells |
|------------|-------------------------------------|-------------|-------------|
| Medium     | 0                                   | 0           | 0           |
| Human IL-5 | 100                                 | 100         | 100         |
| IL-2       | ND*                                 | 2.3 ± 1.3   | 1.2 ± 1.2   |
| IL-3       | 40.0 ± 11.4                         | 3.2 ± 0.5   | 5.7 ± 4.7   |
| IL-4       | 6.2 ± 5.5                           | 4.4 ± 4.4   | 6.7 ± 4.7   |
| IL-6       | 0                                   | ND          | ND          |
| G-CSF      | 125I                              | 0           | ND          |
| GM-CSF     | 59.6 ± 8.3                          | 45.7 ± 9.3  | ND          |
| TNF-α      | 8.2 ± 6.3                           | ND          | ND          |
| IL-1α      | ND                                  | 2.0 ± 0.8   | 5.7 ± 4.7   |
| IL-1β      | ND                                  | 5.7 ± 4.7   | 5.7 ± 4.7   |
| LIF        | 2.4 ± 2.4                           | ND          | ND          |
| COS cell human IL-5 | 100 | ND | ND |
| COS cell mouse IL-5 | 100 | ND | ND |
| COS cell mock | 0 | ND | ND |

* ND, not determined.

To ascertain that the competition for 125I-IL-5 binding to eosinophils by GM-CSF and IL-3 took place at the cell surface, acid elution experiments were performed where after incubation for 1 h at 4 °C eosinophils were resuspended in medium pH = 7.0 or pH = 2.0. In an experiment where the competition by IL-3 was 41.4% and by GM-CSF was 44.6%, 96.5% of cell-associated radioactivity was acid-dissociable. In the presence of IL-5 and GM-CSF, the acid-dissociable counts were 79.0 and 81% of cell-associated radioactivity, respectively. In two other experiments the percent acid-dissociable specific counts in the absence of competitors or in the presence of GM-CSF and IL-3 were 91.4, 85.8, and 85.5, respectively, in experiment one, and 87.7, 100.0, and 94.5, respectively, in experiment two. The fact that the non-acid-dissociable counts were always much less than the percent competition with GM-CSF and IL-3 indicates that the competition observed occurred on the surface of eosinophils.

Given that the binding of 125I-IL-5 was inhibited by GM-CSF and IL-3 it was important to determine whether this competition was unidirectional or whether all three eosinophil hemopoietic growth factors cross-competed for binding. The results using eosinophils from 19 different individuals showed that the binding of 125I-IL-5 was consistently inhibited by GM-CSF and IL-3 with GM-CSF being the stronger competitor (Fig. 4). However, when IL-5 was used as a competitor it inhibited the binding of GM-CSF and IL-3 to eosinophils from only a few donors, and in general this competition was of a smaller magnitude to that seen with GM-CSF or IL-3 (Fig. 4). Similar results were found using HL-60 cells, where GM-CSF consistently inhibited IL-5 binding; however, IL-5 was much less effective at competing for 125I-GM-CSF. The results from three different experiments showed that the binding of 125I-IL-5 was inhibited 45.7 ± 3.3% by GM-CSF and that the binding of 125I-GM-CSF was inhibited 7.8 ± 1.6% by IL-5.

The competition by GM-CSF and IL-3 of 125I-IL-5 binding to eosinophils was further studied by carrying out quantitative inhibition binding experiments. The results showed that the inhibition by GM-CSF and IL-3 was not complete up to a concentration of competitor of 10⁻⁷ M (Fig. 5). Reciprocally, IL-5 only partially inhibited the binding of 125I-GM-CSF and 125I-IL-5 to these eosinophils (Fig. 5). Similar results were obtained with HL-60 cells where GM-CSF competition of 125I-IL-5 binding was only partial up to a concentration of competitor of 10⁻⁷ M (data not shown).

Affinity Cross-linking of the Human IL-5 Receptor—Cross-linking of bound 125I-IL-5 to HL-60 cells by bis-(sulfosuccinimidyl)suberate and analysis by SDS-PAGE demonstrated two major complexes of about 350,000 and 80,000 (Fig. 6). These complexes were not seen in the absence of cross-linker or when excess unlabeled IL-5 was added to the reaction mixture. Importantly, unlabeled GM-CSF almost completely inhibited the formation of both complexes while unlabeled IL-3 did not compete. We have been unable, so far, to obtain cross-linking data from peripheral blood eosinophils under several different experimental conditions. This may be related to the unique composition of these cells in terms of granule contents and variety of proteases.

DISCUSSION

We show here that human eosinophils express high affinity receptors for IL-5 and that the binding of IL-5 is inhibited by the two other eosinophil-active hemopoietic growth factors, IL-3 and GM-CSF. In contrast, neither tumor necrosis factor α, a cytokine that stimulates the function (27) but not the production of human eosinophils, nor IL-4, IL-6, or leukemia inhibitory factor inhibited IL-5 binding. The competition between IL-5, IL-3, and GM-CSF on the surface of eosinophils may be important for understanding the common activation properties of these cytokines. Several studies on eosinophil function have shown that IL-5 as well as IL-3 and GM-CSF enhance eosinophil superoxide production (2, 8, 9), antibody-dependent killing of tumor cells (2, 7–9) and helminths (10–12), degranulation (28), survival in vitro (10–13), conversion from a normodense to a hypodense phenotype (10–12), phagocytosis (2, 7–9), and the biosynthesis of proteoglycans (14). Interactions with a common receptor complex may constitute one unifying mechanism. Other possibilities such as the utilization of a common signal-transduction pathway downstream from the receptor remain to be explored. The second implication of the present findings is that the competition between IL-5, IL-3, and GM-CSF may serve to limit eosinophil stimulation, thus preventing the excessive release of oxygen products, leukotrienes, and granule contents by these cytokines with potentially harmful consequences to the host (29).
Fig. 5. Quantitative inhibition of binding of 125I-IL-5 by GM-CSF and IL-3 (panel A, pool of two experiments) and of 125I-GM-CSF (panel B, pool of three experiments) and 125I-IL-3 (panel C, pool of two experiments) binding by IL-5. The 125I-IL-5, 125I-GM-CSF, and 125I-IL-3 concentrations were 50–80 pM. The bars span 1SD.

Fig. 6. Characterization of the IL-5 receptor by affinity cross-linking. HL-60 cells (1 × 10⁶) were incubated with 125I-IL-5 (1 × 10⁻¹⁸ M) at 4°C for 5 h in the presence of medium alone (lanes a and b) or in the presence of a 500-fold molar excess of unlabeled hIL-5 (lane c), hGM-CSF (lane d), or hIL-3 (lane e). Cells were then harvested, washed, cross-linked, and extracted as described under "Experimental Procedures." Lane a depicts a control sample in which all procedures were conducted except addition of cross-linker. Aliquots were boiled in sample buffer containing 2% SDS and 5% 2-mercaptoethanol and subjected to electrophoresis on an 8% polyacrylamide gel.

Of human primary myeloid cells, only eosinophils and basophils expressed IL-5 receptors. The IL-5 receptors on human blood eosinophils were low in numbers (215–690/cell) and of high affinity (Kₐ = 1.2 × 10⁻⁸ M⁻¹). No low affinity receptors were detected on eosinophils using 125I-IL-5 up to a concentration of 1.4 nM. In two cases where eosinophils from pleural effusions were studied the numbers of IL-5 receptors were very small (Table I). It is not clear whether this represents a true difference between blood and tissue eosinophils or perhaps more likely that these eosinophils have been exposed to IL-5 in vivo, leading to occupation or down-regulation of IL-5 receptors. Although GM-CSF or IL-3 might also occupy IL-5 receptors, the selective accumulation of eosinophils in these two cases makes IL-5 the more likely ligand.

IL-5 also bound to basophils purified from the blood of one patient with chronic myeloid leukemia undergoing basophilic differentiation but not to neutrophils or monocytes. This is consistent with the ability of IL-5 to enhance histamine release from human basophils (30, 31) and with the inability of IL-5 to stimulate or prime neutrophils for the release of superoxide anion and to stimulate monocyte adherence (data not shown). Of the human cell lines studied, IL-5 receptors were only detected on the promyelocytic leukemia HL-60. A subline of HL-60 cells has also recently been shown to express IL-5 receptors after incubation with Na-butyrate (32). Interestingly in view of the capacity of mouse IL-5 to stimulate and to bind to mouse B cells with high affinity (33), high affinity binding sites for IL-5 on human B and pre-B cell lines were not detected.

Mouse IL-5 has been demonstrated to be active on human eosinophils and in fact this cytokine has been widely used on human eosinophils on a variety of functional studies (7, 12, 13, 27). It was, therefore, important to establish whether it bound to the same receptor as human IL-5. The full competition of mouse IL-5 for 125I-human IL-5 binding to eosinophils strongly suggests that this is the case. These results are also relevant for structure-function studies of the IL-5 molecule as they suggest that the binding domains in human and mouse IL-5 are present in the conserved regions of these molecules.

The inhibition of IL-5 binding to eosinophils by IL-3 and GM-CSF was a very consistent finding observed in 12/12 individuals examined. This is in contrast with a recent report mentioning lack of competition; however, no data was shown nor was the bioactivity of the 125I-IL-5 used presented (34). In contrast to IL-3 and GM-CSF, both of which consistently inhibited the binding of 125I-IL-5 to eosinophils (Table II, Figs. 4 and 5) and also compete for each other's binding (18), IL-5 inhibited the binding of 125I-IL-3 and 125I-GM-CSF in only some individuals. The reason for this heterogeneity is not clear but it is unlikely to be due to experimental variation as IL-5 repeatedly inhibited 125I-GM-CSF binding to HL-60 cells very poorly. Whether this reflects different eosinophil subpopulations, or differences in their phenotype (hypodense versus normodense), remains to be seen. Also it is not clear what influence many clinical conditions have on the expression or function of eosinophil IL-5 receptors.

The competition for 125I-IL-5 binding to eosinophils by IL-3 and GM-CSF was partial even when tested at high concentrations of competitors. This may be due to the association of some but not all IL-5 receptors with IL-3 and GM-CSF receptors or to the existence of eosinophil subpopulations expressing either IL-5-specific receptors, or IL-5-, IL-3-, and GM-CSF-associated receptors. Morphological and functional differences have been noted in eosinophils generated in vitro (35) and in vivo (5, 36–38). The existence of eosinophil sub-
populations has also been suggested based on the additive effects of IL-5 and IL-3 and of IL-5 and GM-CSF in stimulating eosinophil formation (39). It would be of great interest to establish whether there are subpopulations of eosinophils capable of binding only to IL-5 or to all three eosinophil hematopoietic growth factors. Autoradiographic studies may ultimately answer this question.

The physical nature of the competition between IL-5, IL-3, and GM-CSF is not yet known, but it may reflect the relatedness of these three eosinophil growth factors and their receptors. The relatedness between IL-5, IL-3, and GM-CSF extends from the structure and close localization of their genes on the long arm of chromosome 5 (40, 41) to the tertiary structure of the mature polypeptides. The IL-5, IL-3, and GM-CSF molecules have conserved features, in particular an area of hydrophobic amino acids in the COOH terminus (42, 43), and their tertiary structure is predicted to be highly conserved (44). While the homology in the COOH terminus could suggest a common binding domain this appears unlikely in view of the pattern of binding and cross-reactivity in primary human myeloid cell types (Table III). It is clear that while in eosinophils the cross-reaction is most evident (18), in monocytes IL-3 and GM-CSF cross-compete (17, 25) but IL-5 is unable to compete, and on neutrophils neither IL-5 nor IL-3 compete for GM-CSF binding (18). It is apparent therefore that for inhibition to occur the homologous receptor needs to be expressed (Table III), and the unique pattern of competition between IL-5, IL-3, and GM-CSF is observed on eosinophils because these cells express all three receptors.

This is supported by the data with HL-60 cells which express GM-CSF but not IL-3 receptors, where GM-CSF but not IL-3 inhibits $^{3}H$-IL-5 binding (Table II) and the formation of the M, 150,000 and 80,000 complexes (Fig. 6).

The inhibition of $^{3}H$-IL-5 binding by IL-3 and GM-CSF may be explained at the receptor level by postulating either steric hindrance or a common receptor complex. In both cases a specific association of IL-5, IL-3, and GM-CSF receptors needs to be invoked. In the first case, the binding of one ligand may physically prevent the binding of a second ligand, while in the second case the receptors for IL-5, IL-3, and GM-CSF may share a polypeptide chain. In this situation IL-5, IL-3, and GM-CSF receptors may be composed of unique binding proteins which are specific for the respective ligands and which become associated with another polypeptide chain that is limiting. This postulate is supported by the recent cloning of a GM-CSF receptor subunit that does not bind GM-CSF but associates with the binding chain of the GM-CSF receptor to provide high affinity binding (46). More experiments with this chain on human eosinophils may reveal one or more mechanisms by which IL-5, IL-3, and GM-CSF exert their common biological activity on these cells.

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References

1. Campbell, H. D., Tucker, W. Q., Hott, Y., Martinson, M. E., Mayo, G., Clutterback, E. J., Sanderson, C. J., and Young, I. G. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6627-6633
2. Lopez, A. F., Sanderson, C. J., Gamble, J. R., Campbell, H. D., Young, I. G., and Vadas, M. A. (1988) J. Exp. Med. 167, 219-224
3. Clutterbuck, E. J., Hirst, E. M. A., and Sanderson, C. J. (1989) Blood 73, 1504-1512
4. Wang, J. M., Rambaldi, A., Biondi, A., Chen, Z. G., Sanderson, C. J., and Mantovani, A. (1989) Eur. J. Immunol. 19, 701-705
5. Owen, W. F., Rothenberg, M. E., Petersen, J., Weller, P. F., Silberstein, D., Sheffer, A. L., Stevens, R. L., Soberman, R. J., and Austen, K. F. (1988) J. Exp. Med. 170, 343-348
6. Owen, W. Jr., Petersen, J., Sheff, D. M., Folkerth, R. D., Silberstein, D., Corson, J. M., Sheffer, A. L., and Austen, F. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8647-8651
7. Lopez, A. F., Begley, C. G., Williamson, D. J., Warren, D. J., Vadas, M. A., and Sanderson, C. J. (1986) J. Exp. Med. 163, 1095-1099
8. Lopez, A. F., Williamson, D. J., Gamble, J. R., Begley, C. G., Harlan, J. M., Klebanoff, S. J., Waltersdorph, A., Wong, G., Clark, S. C., and Vadas, M. A. (1986) J. Clin. Invest. 78, 1220-1226
9. Lopez, A. F., Dyson, P. G., To, L. B., Elliott, M. J., Milton, S. E., Russell, J. A., Jutte, C. A., Yang, Y.-C., Clark, S. C., and Vadas, M. A. (1988) Blood 72, 1797-1804
10. Owen, W. F. Jr., Rothenberg, M. E., Silberstein, D. S., Gasson, J. C., Stevens, R. L., Kusseben, K. F., and Soberman, R. J. 1987. J. Exp. Med. 166, 129-141
11. Rothenberg, M. E., Owen, W. F. Jr., Silberstein, D. S., Woods, J., Soberman, R. J., Austen, K. F., and Stevens, R. L. (1988) J. Clin. Invest. 81, 1986-1992
12. Rothenberg, M. E., Petersen, J., Stevens, R. L., Silberstein, D. S., McKenzie, D. T., Austen, K. F., and Owen, W. F. Jr. (1989) J. Immunol. 143, 2011-2016
13. Begley, C. G., Lopez, A. F., Nicola, N. A., Warren, D. J., Vadas, M. A., Sanderson, C. J., and Metcalf, D. (1988) Blood 68, 162-166
14. Rothenberg, M. E., Pomerantz, J. L., Owen, W. F., Jr., Avraham, S., Soberman, R. J., Austen, K. F., and Stevens, R. L. (1988) J. Biol. Chem. 263, 13901-13908
15. Vadas, M. A., David, J., Butterworth, A., Tinari, N. T., and Siongok, T. A. (1978) J. Immunol. 122, 1228-1228D
16. Hopp, T. P., Prickett, K. S., Price, V. L., Libby, R. T., March, C. J., Cerretti, D. P., Urdal, D. L., and Conlon, P. J. (1986) Biotechnology 6, 1204-1210
17. Park, L. S., Friend, D., Price, V., Anderson, D., Singer, J., Prickett, K. S., and Urdal, D. L. (1989) J. Biol. Chem. 264, 5420-5427
18. Lopez, A. F., Eglinton, J. M., Gillis, D., Park, L. S., Clark, S., and Vadas, M. A. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7922-7926
19. Contreras, M. A., Bale, W. F., and Spar, I. L. (1983) Methods Enzymol. 92, 277-292
20. Calvo, J. C., Radicella, J. P., and Charreau, E. H. (1983) Biochem. J. 212, 259-264
21. Power, S., Kronheim, S., March, C., Hopp, T., Conlon, P., Gillis, S., and Urdal, D. L. (1985) J. Exp. Med. 162, 501-515
