Interleukin-1 (IL-1) plays a major role in the response to infection, inflammation, and immunological challenge. Eosinophils participate in the host response to parasitic infection and allergic and hypersensitivity diseases. The role of IL-1 in these disease states has not been extensively explored. We have reported that purified human monocyte derived IL-1 (mIL-1), a mixture of the two IL-1 forms but predominantly consisting of IL-1 beta, modulates eosinophil oxidative metabolism and enzyme secretion. Although the two major species of IL-1 (IL-1 alpha and IL-1 beta) have identical specific activities on T cells, we now report the selective effects of human recombinant IL-1 (hrIL-1) alpha and hrIL-1 beta on eosinophil function. Whereas hrIL-1 beta caused a significant increase in arylsulfatase secretion (235.4 ± 29% of resting secretion, P ≤ .01) and beta-glucuronidase secretion (135.8 ± 9.6% of resting secretion, P ≤ .02) similar to our experience with mIL-1, hrIL-1 alpha had no effect on enzyme secretion. However, a mixture of hrIL-1 alpha and hrIL-1 beta reproduced the ability of mIL-1 to inhibit the oxidative response to suboptimal doses of phorbol myristate acetate (PMA). When eosinophils were separated into subpopulations by density gradients, we found that eosinophil responses to IL-1 differed among the populations. These results suggest that eosinophil subpopulations respond selectively to each form of IL-1.

INTERLEUKIN-1 (IL-1) is a family of molecules with various biological functions. Originally described as endogenous pyrogen, lymphocyte activating factor, or leukocytic endogenous mediator, IL-1 effects numerous leukocyte cytocidal endogenous pyrogen, lymphocyte activating function and allergic and hypersensitivity diseases. The role of IL-1 in these disease states has not been extensively explored. We have reported that purified human monocyte derived IL-1 (mIL-1), a mixture of the two IL-1 forms but predominantly consisting of IL-1 beta, modulates eosinophil oxidative metabolism and enzyme secretion. Although the two major species of IL-1 (IL-1 alpha and IL-1 beta) have identical specific activities on T cells, we now report the selective effects of human recombinant IL-1 (hrIL-1) alpha and hrIL-1 beta on eosinophil function. Whereas hrIL-1 beta caused a significant increase in arylsulfatase secretion (235.4 ± 29% of resting secretion, P ≤ .01) and beta-glucuronidase secretion (135.8 ± 9.6% of resting secretion, P ≤ .02) similar to our experience with mIL-1, hrIL-1 alpha had no effect on enzyme secretion. However, a mixture of hrIL-1 alpha and hrIL-1 beta reproduced the ability of mIL-1 to inhibit the oxidative response to suboptimal doses of phorbol myristate acetate (PMA). When eosinophils were separated into subpopulations by density gradients, we found that eosinophil responses to IL-1 differed among the populations. These results suggest that eosinophil subpopulations respond selectively to each form of IL-1.

Eosinophils (EOS) are important participants in the host response to parasitic infection and allergic disease. EOS function is enhanced by crude mononuclear cell supernatants that contain "eosinophil activating factor" activity. Stimulated monocytes release products that enhance calcium ionophore-induced leukotriene generation and antibody-dependent cytotoxicity to schistosomula. Among those well-characterized macrophage/moноcyte products that modulate EOS function are granulocyte macrophage colony stimulating factor (GM-CSF), platelet activating factor (PAF), tumor necrosis factor (TNF), and IL-1. GM-CSF prolongs EOS survival and enhances EOS cytotoxicity, leukotriene LTC4 synthesis, and phagocytosis. PAF enhances leukotriene LTC4 synthesis and chemiluminescence and is a chemotactic and chemokinetic stimulus for EOS. TNF possesses EOS activating activity as well as cytotoxicity enhancing activity. Relevant to activation of EOS function by these cytokines is the demonstration that IL-1 induces production of GM-CSF, PAF, and TNF.

In addition, our previous work has demonstrated that monocyte-derived IL-1 (mIL-1) enhances release of granular enzymes while inhibiting ionophore-stimulated release as well as the oxidative response to suboptimal doses of phorbol myristate acetate (PMA). IL-1 alpha and IL-1 beta, two distinct gene products found in mIL-1, produce similar effects on cultured murine thymocytes and cultured human connective tissue cells and bind to the same putative receptor on a variety of cell types. As an extension of our previous observations, we explored the effects of human recombinant IL-1 (hrIL-1) alpha and hrIL-1 beta on superoxide production as a measure of oxidative metabolism as well as on the release of granular enzymes arylsulfatase and beta-glucuronidase. Since functional heterogeneity exists among EOS populations, we examined the response of EOS subpopulations to IL-1. Our results demonstrate that, in contrast to previous cell types examined, IL-1 alpha and IL-1 beta have selective effects on EOS function. Furthermore, functional heterogeneity exists in the response of EOS to these monokines.

MATERIALS AND METHODS

Reagents. Highly purified recombinant hrIL-1 alpha and hrIL-1 beta expressed in Escherichia coli and purified to homogeneity assessed by silver staining and N-terminal amino acid sequencing were the gift of Biogen (Cambridge, MA). As shown in Fig 1, these preparations were equivalent in biological activity in D10.G4.1 cells assayed as previously described. The IL-1 (10 μg/mL) was stored at -70°C in 0.1% bovine serum albumin (BSA) and diluted in phosphate buffered saline (PBS) immediately before use. There was no evidence of endotoxin contamination by the limulus assay. Other biologically active products used for enzyme secretion included platelet activating factor C4 (PAF; Boehringer Mannheim, West Germany), n-formyl-methionyl-leucine-phenylalanine (F-met-leu-phe; Sigma Chemical Co, St Louis), and tumor necrosis factor (TNF alpha; Genentech, Inc, South San Francisco).

Leukocytes. Normal human EOS were purified on discontinuous metrizamide gradients (Accurate Chemical Co, Westbury,
DIFERENTIAL EFFECTS OF IL-1 ON EOS

Fig 1. Effect of hrIL-1 alpha and hrIL-1 beta on D10.G4.1 cells. Bioassay is the standard comitogenic IL-1 assay, as previously described. Control is 1 μg/mL of PHA without IL-1.

minutes at 4°C. An aliquot was reserved for enzyme assay as described.

Enzyme assays. Beta glucuronidase release was assayed using 4-methylumbelliferyl beta-D-glucuronide (Sigma) in a 0.1 mol/L sodium acetate buffer, pH 4.8, as previously described. Arylsulfatase B release was assayed using 4-methylumbelliferyl sulfate (Sigma) in a 0.2 mol/L sodium acetate buffer, pH 5.7. Fluorescence was determined at excitation 365 nm and emission 450 nm. Calculations for percent enzyme release were performed as previously described. Lactate dehydrogenase (LDH) was used as a marker for cell disruption according to the methods of Bergmeyer et al.

Binding studies. IL-1 binding studies were performed as previously described by Rosoff et al. Briefly, recombinant IL-1 alpha or IL-1 beta were radio-iodinated by a modified chloramine T method to a specific activity of 90 μCi/μg. Binding of 125I-IL-1 was measured in triplicate assays using 1 x 106 EOS suspended in 0.15 mL of RPMI 1640 with 1.0% BSA and 20 mmol/L HEPEES (pH 7.2) and 6.5 x 10−8 mol/L 125I-IL-1. Nonspecific binding was determined in the presence of excess unlabeled rIL-1. Free 125I-IL-1 was separated from cell-bound 125I by centrifugation of 0.05 mL of the reaction mixture through 200 μL of silicon oil for 30 seconds in a microfuge. The tube tip containing the cell pellet was removed and radioactivity measured in a gamma counter.

RESULTS

Superoxide production. Our initial experiments examined the effects of hrIL-1 on oxidative metabolism in resting EOS as measured by superoxide anion production. Neither hrIL-1 alpha nor hrIL-1 beta at concentrations from 10 ng/mL to 100 ng/mL affected superoxide production (data not shown). The addition of either hrIL-1 alpha or hrIL-1 beta did not affect the oxidative response to suboptimal doses of PMA (100 pg/mL). However, when hrIL-1 alpha (1 pg/mL) and hrIL-1 beta (100 pg/mL) were added together and preincubated with EOS for 30 minutes before the addition of PMA, there was a significant inhibition of the response to suboptimal doses of PMA (Table 1), thus confirming our previous observations. Other combinations of hrIL-1 alpha (10 ng/mL to 1 pg/mL) and hrIL-1 beta (10 ng/mL to 100 pg/mL) did not affect PMA-induced superoxide production (data not shown).

Release of granular enzymes. mL-1 stimulates release of granular enzymes from EOS. In the present studies, we examined separately the actions of hrIL-1 alpha and hrIL-1 beta. hrIL-1 alpha had no effect on either arylsulfatase or beta-glucuronidase release over the wide range of concentrations tested (Fig 2A). Increasing the incubation time to three

Table 1. Effect of IL-1 Alpha and IL-1 Beta on PMA Stimulated O2− Production

<table>
<thead>
<tr>
<th>IL-1 Addition</th>
<th>% PMA Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>IL-1 beta 100 pg/mL</td>
<td>104.2 ± 1.8</td>
</tr>
<tr>
<td>IL-1 alpha 1 pg/mL</td>
<td>106.3 ± 3.8</td>
</tr>
<tr>
<td>IL-1 beta 100 pg/mL</td>
<td></td>
</tr>
<tr>
<td>+ IL-1 alpha 1 pg/mL</td>
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</table>

Results are expressed as percent of PMA (100 pg/mL) stimulation. PMA alone reduced 5.48 ± 1.37 nm cytochrome C (mean ± SEM, n = 3). Eosinophils were preincubated for 30 minutes with IL-1 before the addition of PMA and cytochrome C.
When hIL-1 alpha and hIL-1 beta were added together, there was no difference from the response of hIL-1 beta alone (Fig 3).

Other biologically active substances that modulate EOS function were examined for additive enhancing or inhibitory effects. hIL-1 beta was tested in the presence of TNF (concentrations from 1 pg/mL to 1 ng/mL), PAF (10^-6 mol/L to 10^-10 mol/L) and F-met-leu-phe (10^-4 to 10^-8 mol/L). However, no differences were noted (data not shown).

**Enzyme secretion with different EOS populations.** EOS of different densities have different functional capabilities as indicated by their ability to kill antibody-coated schistosomula. Previous studies of patients with marked hyper eosinophilia have compared pooled EOS harvested above the 23% metrizamide layer (hypodense EOS) with pooled EOS harvested from more dense metrizamide layers (normodense EOS). In order to discover whether heterogeneity is present between normal EOS of different density, we examined the responses of lighter density EOS in comparison with intermediate and heavier density EOS from the same normal donor. EOS were separated into three subpopulations on metrizamide gradients: the 22/23 interface (layer 23), the 23/24 interface (layer 24), and the 24/25 interface (layer 25). EOS purity was >90% for each layer in these studies. hIL-1 alpha and hIL-1 beta were tested on these subpopulations. Comparisons were made between subpopulations. There were no differences in resting arylsulfatase and beta-glucuronidase release between the layers. hIL-1 alpha (1 ng/mL and 100 pg/mL) caused no modulation of arylsulfatase secretion in any cell population studied. hIL-1 beta (1 ng/mL and 100 pg/mL) caused a significant increase in arylsulfatase and beta-glucuronidase release in layer 24 (Fig 4B). Layer 24 was more responsive to stimulation of arylsulfatase release by hIL-1 beta than was layer 23.

**IL-1 binding studies.** IL-1 alpha and IL-1 beta are believed to share a common receptor. The differential effects of IL-1 alpha and IL-1 beta observed here raised the question of whether EOS might have separate receptors for IL-1 alpha and IL-1 beta. Binding of 125I-IL-1 alpha and 125I-IL-1 beta was studied as described in Materials and Methods. One x 10^6 EOS were incubated at 4°C with 125I labeled IL-1 alpha or IL-1 beta and nonspecific and specific binding determined. No significant differences were observed, suggesting that specific receptor binding does not occur. Furthermore, the addition of unlabeled IL-1 alpha or IL-1 beta did not significantly alter binding of 125I-IL-1 (data not shown), confirming a lack of detectable receptors. That this was not due to altered reactivity of radiolabeled 125I-IL-1 is suggested by binding of the labeled material to EL-4 cells, as has been previously published. Four experiments were performed in triplicate, all of which failed to demonstrate specific binding of IL-1 alpha or IL-1 beta. The limits of detection of this assay are such that receptor numbers less than 200 per cell would not be detected. Full Scatchard analysis using multiple concentrations of IL-1 could not be performed because of the large number of EOS required (>4 x 10^6).

**DISCUSSION**

Although IL-1 acts directly on many immunocompetent cells, its role in parasitic and hypersensitivity diseases has not
Differential Effects of IL-1 on EOS

The effect of hIL-1 alpha and hIL-1 beta on different subpopulations of eosinophils. Eosinophils were incubated for 90 minutes with IL-1. Three experiments were performed in triplicate and are expressed as percent of unstimulated enzyme release. Statistics are calculated for the differences between layers 23, 24, and 25.

(A) Effect of hIL-1 alpha. IL-1 alpha did not alter arylsulfatase or beta-glucuronidase release of tested eosinophil populations. (B) Effect of hIL-1 beta. IL-1 beta caused significantly more arylsulfatase release in layer 24 than in layer 23 (P < .05). Beta glucuronidase release is significantly greater than resting release only in layers 23 or 24 (P < .05).

been extensively explored. Our present studies demonstrate a selective modulation of EOS function in vitro by IL-1 and suggest a possible role for IL-1 in parasitic infection and/or hypersensitivity disease. It has been reported that the two major species of IL-1 (IL-1 alpha and IL-1 beta) bind to the same receptor on several cell types and, despite <26% amino acid homology, these two molecules share similar biological properties. Thus, we have explored the effects of these monokines on specific EOS functions (enzyme release and oxidative metabolism) on mixed and fractionated cell populations.

In our previous work, we reported that mMIL-1 inhibited the oxidative response to suboptimal doses of PMA. In the present study, neither hIL-1 alpha nor hIL-1 beta individually caused modulation of this response. We speculate that this result may have been due to the combined effects of IL-1 alpha and IL-1 beta present in mMIL-1. In fact, we observed the inhibition of PMA-induced superoxide production when the two cytokines were added together. However, the modest though statistically significant change may indicate the presence of additional factors influencing EOS function in monocyte supernatants. We also confirmed our previous observation by showing that hIL-1 beta causes secretion of the granular enzymes arylsulfatase and beta-glucuronidase. Although more arylsulfatase was released than beta-glucuronidase, since these enzymes are found in different granules in the EOS, it is not surprising that they are susceptible to differential modulation. hIL-1 alpha did not cause secretion either individually or in combination with hIL-1 beta. Thus, we conclude that the effect seen with mMIL-1 is probably due to IL-1 beta. Though the absolute amount of enzyme release is modest, the reproducible effects (a total of over 20 experiments) confirm the validity of these observations. Additionally, since release may be modified by the orientation of the cells in tissues or the presence of other stimuli, it is possible that the degree of release is higher at focal sites in tissues. That IL-1 acts to selectively enhance other signals is confirmed by studies of IgG and IgE-mediated release.

Hypodense EOS have been shown to exhibit increased cytotoxicity for larvae of Schistosoma mansoni, and increased oxygen consumption. However, considerable heterogeneity exists between EOS from patients with different origins of EOS and those from the same hypereosinophilic donor. These hypodense EOS, harvested from patients with marked peripheral blood hypereosinophilia, are prepared by pooling EOS from metrizamide layers <23%. Since they copurify with neutrophils that are of similar density, it is difficult to obtain pure populations for metabolic studies. More importantly, our interests were to demonstrate that heterogeneity exists between "normal" EOS of varying density. The studies reported here suggest that normal EOS from both normal and mildly hypereosinophilic donors constitute a functionally heterogeneous population. Intermediate density EOS of layer 24 are more responsive to modulation by hIL-1 beta than are EOS of other density from the same donor. Since our EOS subpopulations were >90% pure, we do not attribute the differential response to neutrophil contamination. Additionally, neutrophils have been shown to be unresponsive to IL-1 and do not contain significant arylsulfatase. These findings may be important factors in studying EOS function since, in a mixed population of cells, certain populations may be responding to a stimulus while other populations may not. Thus, a high degree of control and selectivity in modulation of EOS function may be present. These studies also suggest that the maturation or pre-existing state of the EOS contributes to its ability to respond to the monokines IL-1 alpha and IL-1 beta. This observation may extend to the actions of other EOS stimuli.

Our studies show that, despite their identical specific activities on D10.G4.1 cells, hIL-1 alpha and hIL-1 beta produce different effects on EOS function. These studies also confirm our previous observations that IL-1 modulates EOS function but that activation of one cellular function does not necessarily activate all functions. Our results raise the possibility that either IL-1 alpha and IL-1 beta signal for different
second messengers or that IL-1 alpha or IL-1 beta have different receptors. Recent evidence presented by Rosoff et al.\(^2\) establishes that Jurkat cells that lack detectable receptors for IL-1 respond to IL-1 by increasing diacylglycerol and phosphorylcholine production from phosphatidylcholine in the absence of increased phosphatidylinositols turnover.

We speculate that EOS might have a similar novel receptor-independent mechanism of IL-1 action, the nature of which remains to be precisely defined. However, it is also possible that a more sensitive technique for measurement of receptors would detect receptors for IL-1 alpha and IL-1 beta.

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Differential effects of interleukin-1 alpha and interleukin-1 beta on human peripheral blood eosinophils

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