Eosinophil Activation by Colony-Stimulating Factor in Man: Metabolic Effects and Analysis by Flow Cytometry

By M. A. Vadas, G. Varigos, N. Nicola, S. Pincus, A. Dessein, D. Metcalf, and F. L. Battye

Substantial increases in the killing capacity of human eosinophils after in vitro incubation with human placental conditioned medium (HPCM), a standard source of colony-stimulating factor (CSF), have recently been described. In this article, the interaction between HPCM and purified human eosinophils is analyzed by flow cytometry and by effects on iodination, superoxide production, and protein synthesis. HPCM increased the intensity of natural eosinophil autofluorescence (aFlu) at 460 nm after the absorption of ultraviolet light (360 nm) in a manner that was both time- and dose-dependent. Measured in arbitrary units, eosinophil aFlu was 72 ± 7.3 (arithmetic mean ± SEM) and 121 ± 3.2 after 18-hr incubations in the absence or presence of HPCM, respectively. The activity in HPCM responsible for these changes chromatographed on Ultragel AcA44 columns with CSF and with the less hydrophobic variant of CSF (CSF-α) on phenyl Sepharose. Mouse spleen, but not mouse lung, conditioned medium was also active on human eosinophils in this assay. Both CSF-α and mouse spleen conditioned medium also contain eosinophil colony-stimulating activity (CSA), whereas inactive CSFs with no effect on mature eosinophils, CSF-β, and mouse lung conditioned medium also lack eosinophil CSA. CSF-α stimulated superoxide production of resting eosinophils (from 0.03 ± 0.03 to 0.47 ± 0.08 nmoles cytochrome-c reduced/10^6 eosinophils) and of eosinophils incubated with preopsonized zymosan (from 0.15 ± 0.06 to 0.73 ± 0.07). It also stimulated iodination by resting eosinophils (from 0.76 ± 0.16 to 2.60 ± 0.72 nmoles I/10^6 eosinophils/hr) and of eosinophils incubated with preopsonized zymosan (from 7.52 ± 2.08 to 29.8 ± 1.32). In contrast, CSF-β was inactive in these assays. CSF-α also stimulated, between 2- and 15-fold, the new protein synthesis of eosinophils. Thus, substances that stimulate the differentiation of progenitor cells into eosinophils also interact with peripheral mature eosinophils, and the activation of postmitotic cells may be a physiologic role of CSF-like molecules.

Eosinophil-mediated destruction of parasites has been suggested to be an important mechanism of protective parasite immunity. However, eosinophils differ among individuals; for example, there is a direct correlation between the degree of eosinophilia in an individual and the capacity of eosinophils from that individual to kill parasites. Eosinophils from eosinophilic patients also have a decreased surface charge as well as evidence of increased metabolic activity. This "activation" of eosinophils observed in vitro presumably also reflects an in vivo variation in their toxic potential. Eosinophil activation may therefore be an important component of host defense against parasites.

Recently, it was shown that eosinophil activation can also take place in vitro. Human placental conditioned medium (HPCM) increased by fivefold the capacity of eosinophils to kill schistosomula of Schistosoma mansoni in the presence of subthreshold amounts of antibody or complement. The magnitude of this effect was greater than that of other agents that alter eosinophil function. It was also found that a molecule with similar properties to colony-stimulating factor (CSF) was the active substance in HPCM. Since CSF is probably involved in the development of granulocytosis, it is also possibly a substance involved in the development of eosinophilia and therefore is now a candidate for the substance responsible for the eosinophil activation seen in eosinophilia.

The in vitro activation of human eosinophils by HPCM has two important implications. First, the in vivo activation of eosinophils seen in parasite-induced eosinophilia can now be explained as a peripheral activation of mature cells. Second, since activated eosinophils kill well with subthreshold amounts of antibody or complement, they can compensate for deficiencies of other parts of the host’s specific or nonspecific immune response. Thus, agents that lead to eosinophil activation have therapeutic potential.

In this article we analyze eosinophil activation by flow cytometry using the fluorescence-activated cell sorter (FACS). Characteristic changes develop in eosinophils in a time- and dose-dependent fashion after exposure to HPCM and consist of an increase in eosinophil autofluorescence (aFlu). A subspecies of human CSF, CSF-α, appears to be responsible for these effects, and eosinophils exposed to CSF-α also have an increased rate of protein synthesis, superoxide production, and iodination compared to controls, sug-
gesting that CSF-α is a powerful modulator of the function of mature eosinophils.

**MATERIALS AND METHODS**

**Media and Chemicals**

Minimal essential medium (MEM) (GIBCO, Grand Island, NY) and MEM supplemented with 10% fetal calf serum inactivated at 56°C for 1 hr, 100 U of penicillin G, 100 μg/ml streptomycin (Microbiological Associates, Bethesda, MD), 1% glutamine, 25 mM HEPES buffer, and 30 mg/liter DNase (Worthington Biochemical Corporation, Freehold, NJ, 240 U/mg) (MEM/FCS) was used for most incubations. RPMI 1640 Select-Amine kit (GIBCO) was used for the measurement of uptake of labeled amino acids. L-(14C) methionine (1120 Ci/m mole) was obtained from the Radiochemical Centre, Amersham, U.K. Lipo polysaccharide (LPS) (Sigma, St. Louis, MO) was diluted as indicated. Human toxicity phosphate-buffered saline (PBS) contained 0.02 M sodium phosphate buffer in 0.149 M NaCl.

**Donors of Eosinophils**

Patients (3 with eczema, 3 with carcinoma of the lung, 1 with polycythemia vera, 1 with cirrhosis, 1 with multinodular goiter, 1 with parasite infestation) with eosinophilia (700-2,000/cu mm) or normal donors known to have slightly elevated eosinophil counts (700-1,200/cu mm) were used. In all instances, informed consent was obtained in writing before blood was taken.

**Preparation of Human Eosinophils**

This is described in detail elsewhere. Briefly, peripheral blood was collected into heparinized syringes and red cells sedimented with dextran (T500 Pharmacia, Sweden). The leukocytes were washed in MEM/FCS and eosinophils purified by centrifugation over discontinuous, slightly hypertonic, metrizamide gradients. The purity of eosinophils (excluding contaminant red cells) in these experiments ranged from 85% to 100% (mean 94.7% ± 4.9%). Since residual red cells copurify with eosinophils in these gradients, they were lysed by suspending the cell pellet in 0.168 M NH4Cl for 10 min at room temperature and then washing the cells 3 times in PBS supplemented with 5% FCS.

**Incubation of Eosinophils**

Eosinophils were resuspended at 10⁶ cells/ml and 450 μl dispensed into sterile plastic tubes (2001 FALCON, Becton Dickinson, Cockeysville, MD). To these, either 50 μl of HPCM or one of its purified fractions or control solution was added. The concentration of these substances, unless otherwise stated, was adjusted to their maximal colony-stimulating capacity. When drugs or radioactive substances were used, 400 μl of eosinophils were dispensed and 2 lots of 50 μl of experimental substances added to this. The mixtures were incubated for various periods at 37°C in 5% CO₂. The cells were washed once in 5% FCS in PBS and once again before analysis on the FACS.

**Analysis and Separation Using Light Scatter and Autofluorescence**

A Becton Dickinson FACS II cell-sorting instrument with a tunable Argon ion laser (Spectra Physics, model 164) was used. This instrument had been modified to allow the use of all three measured parameters for cell sorting and was interfaced to a computer data processing system. Computer interface schematics and data handling software were kindly communicated by Robert J. Romanoff of the National Institutes of Health and Dr. Gary Salzmann of Los Alamos Scientific Laboratories. The laser was operated at the 351.1 nm and 363.8 nm emission lines at 20 mW total output power. 25,000 or 50,000 particles were analyzed, and 3 parameters were regularly measured: (1) "0°" light scatter (O°Sc) (light collected in a cone between I° and 20° of the laser direction), (2) "90°" light scatter (90°Sc) (light collected between 60° and 120° from the laser direction), and (3) cellular autofluorescence (aFlu) with peak intensity at 460 nm, which was separated from the "90°" light scatter using a 425 nm long-pass optical filter. The instrument was calibrated daily with 1.83 μm fluorescent standard beads (Polysciences, Warrington, PA). The cell-sorting rate was always less than 3,500 cells/sec, and deflected cells were collected into 50% FCS in PBS in siliconized sterile glass tubes. The 0°Sc of cells is a useful measure of size when incident light is in the visible range, but at 360 nm, this parameter is a more complex function of cell structure. It was used in the present work only to electronically exclude small subcellular particles from analysis. Each of the three measured parameters was stored in a linear array of 256 memory channels, the channel number then representing an arbitrary unit for use in analysis and sorting.

**Cell Counts and Differentials**

Cell counts and viabilities were determined by an eosin dye exclusion method. For differential counts, 1 drop of approximately 0.1-1 × 10⁶ cells/ml cell suspension in 50% FCS was placed in a Shandon-Elliot cytacentrifuge and spun onto alcohol-cleaned slides (1,500 rpm, 10 min). The slides were then air-dried, fixed in methanol, and stained with Giemsa. Differential counts were performed at 1,000× magnification counting 100-200 cells.

**Bone Marrow Culture and Morphology**

The soft agar technique was used, and full details are published elsewhere.

**Preparation of Conditioned Media**

HPCM was prepared as described previously. Briefly, pieces of fresh human placenta were incubated for 7 days in RPMI 1640 medium containing 5% fetal calf serum. The supernatant was then collected, pooled, and tested for CSA.

**Purification of CSF From HPCM**

(A) Gel filtration on Ultrogel- AcA44. HPCM was concentrated 10-fold using an Amicon DC-2A apparatus with an H1P10 hollow-fiber cartridge and dialyzed against distilled water. It was then absorbed to calcium phosphate gel and eluted with 0.05 M sodium phosphate buffer as described previously. This concentrated material (25 ml) was then applied to a column of Ultrogel-AcA44 (LKB-Produkter, Bromme, Sweden) 2.6 × 100 cm and eluted at a flow rate of 15 ml/hr with phosphate-buffered (0.02 M, pH 7.3) saline (0.15 M) containing polyethylene glycol 6000 (0.005% w/v). Fractions of 5 ml were collected and assayed separately before pooling.

(B) Fractionation on phenyl Sepharose CL-4B. Samples of HPCM (either calcium phosphate-seated or active fraction gel filtration) were applied to a column of phenyl Sepharose CL-4B (2.6 × 20 cm) (Pharmacia Fine Chemical, Uppsala, Sweden) equilibrated in phosphate-buffered saline. The column was eluted with the same buffer until eluate absorbance reached background level and then the eluate was changed to 60% (v/v) ethylene glycol in distilled water. CSF failing to bind to the resin in phosphate-
buffered saline was designated CSF-α, and that eluting with ethylene glycol designated CSF-β.  

**Superoxide (O₂⁻) Production**

Purified eosinophils (10⁷/ml) in Hank's balanced salt solution were added to duplicate 15 × 75 mm plastic tubes with 100 nmole cytochrome-c (type VI, Sigma) and O₂⁻ production measured by the reduction of cytochrome-c observed at 550 nm.¹⁶ where indicated, 1 μg/ml of phorbol myristate acetate (PMA, Sigma) or 0.5 ml preopsonized zymosan (Sigma) was used to stimulate O₂⁻ production. Incubations were performed at 37°C in a stationary water bath and terminated by rapid cooling to 4°C and centrifugation at 4°C at 1,000g for 10 min. In control experiments, CSF-α and β without cells did not reduce cytochrome-c, and superoxide dismutase completely inhibited PMA or CSF-α-stimulated increases in O₂⁻ production.

**Quantitative Leukocyte Iodination**

This is described in detail elsewhere.²⁵ Briefly, reaction mixtures contained 10⁸ eosinophils, 0.004 M sodium phosphate buffer, 0.128 M NaCl, 0.14 M KCl, 0.001 M MgCl₂, and 40 nmole ¹²⁵I (0.05 μCi ¹²⁵I) (New England Nuclear, Boston, MA). Incubations were terminated after 1 hr by the addition of cold 10% trichloroacetic acid. Precipitates were washed 4 times and the number of nmole ¹²⁵I incorporated/10⁶ leukocyte/hr determined.

**Measurement of Protein Synthesis**

The incorporation of l-(³⁵S)-methionine into eosinophils was determined after 4-18-hr incubation in methionine-free RPMI 1640-FCS (GIBCO). The cells were then washed 3 times in PBS, lysed in 0.5% Triton X-100, and beta-emission was counted either directly or after 3 serial precipitations with 10% trichloroacetic acid (TCA).

**Measurement of Red Cell Lysis**

Leukocyte-free red cells were obtained as a by-product of the eosinophil separation procedure from the pellet of the metrizamide gradients. Of packed red cells, 0.3 ml were then mixed with 0.2 ml of 300 μCi/ml ⁵¹Cr (Amersham, U.K.), diluted to 1.0 ml, and incubated at 37°C for 0.5 hr. They were then washed 3 times in PBS and 50 μl of a 10⁻¹³/ml solution added to 450 μl mixture of eosinophils and CSF. The amount of ⁵¹Cr released into the supernatant was then measured after 18 hr incubation.

**RESULTS**

**Flow Cytometric Characteristics of Human Eosinophils**

Eosinophils, purified on discontinuous metrizamide gradients, were analyzed on the FACS immediately or after 18-hr incubation at 4°C. There was no observable difference between these two types of samples. Analysis of the 460-nm emission of the cells (aFlu) showed characteristic profiles, a representative example of which is shown in Fig. 1a, where the number of cells (ordinate) is plotted against aFlu channel numbers (abscissa). There are three bands with notable features: band A has low (< channel 10), band B intermediate (channels 10–50), and band C high (> channel 50) aFlu. Further analysis showed particles in band A to have low intensity 90°Sc, and microscopic analysis after sorting showed them to be red cells. Further proof for this band being red cells was that purified red cells had identical FACS profiles to the particles found in band A and that lysis of red cells in the original samples caused the disappearance of this band. Cells in band B, with intermediate aFlu (10–50), showed high intensity 90°Sc and consisted of viable eosinophils but with poorly staining nuclei. Cells in band C had high aFlu (>50) and high intensity 90°Sc and were viable eosinophils. These analyses were repeated 5 times, and although the relative heights of the various peaks varied slightly, the pattern remained constant.

**Effect of Incubation of Eosinophils at 37°C**

Figure 1b illustrates the aFlu profiles of eosinophils incubated for 18 hr at 37°C. Band A remained unaltered, band B increased in size and band C decreased in size in comparison with cells incubated at 4°C, but analysis of 90°Sc and morphological features failed to identify any differences between incubations at 4°C and 37°C. In addition to the decrease in the number of cells in band C, the aFlu of these cells was also less than band C particles in the 4°C incubation. In 5 experiments, 65.1% ± 8.6% (arithmetic mean ± 1 SEM) of 4°C incubated cells and 45.2% ± 5.4% of 37°C incubated cells had aFlu greater than channel 100.

**Effect of Incubation of Eosinophils at 37°C With HPCM**

HPCM, at a concentration that was maximally stimulatory in a colony-forming assay, was added to eosinophils incubated at 37°C. There was no difference in the viability or in the recovery of cells incubated in the presence or absence of HPCM (viability >96% and recovery >80%) during the 18-hr incubations. It should be noted, however, that after more prolonged (40 hr) incubations, the viability of eosinophils without HPCM fell (36% ± 15%), whereas that of HPCM-incubated eosinophils was better maintained (81% ± 6%, p < 0.05). Figure 1c illustrates the aFlu profiles of eosinophils incubated for 18 hr at 37°C with HPCM. All three bands show changes.

Band A disappeared. It is likely that the red cells were lysed, since addition of ⁵¹Cr-labeled red cells to the incubations showed release of label in preparations containing HPCM and eosinophils but not with either agent alone (988 cpm, 1,030 cpm, 1,217 cpm, and 5,355 cpm of ⁵¹Cr was released in incubations with red cells alone, red cells plus eosinophils, red cells plus HPCM, and red cells plus eosinophils plus HPCM, respectively).

The proportion of particles in band B in comparison with band C was unaltered; however, the 90°Sc of >95% of these particles was now less than channel 20,
and sorting showed that most of these particles appeared to be free eosinophil granules. Most of the cells were now found in band C. In addition, these cells had higher aFlu than cells incubated at 37°C without HPCM. In demonstrating this effect, it is important to consider that the FACS analyzes a constant number of particles that are not necessarily granulocytes. To eliminate this problem, lysis of red cells before incubation is helpful, but the most satisfactory method is restricting analysis to particles with high 90°Sc (i.e., granulated or multilobed cells and excluding free granules and red cells). This manipulation is shown in Fig. 2a, where the data in Fig. 1b and c are reanalyzed. In order to facilitate the expression of these results, cumulative curves are drawn (Fig. 2b), and multiple experiments can be compared by noting either the proportion of cells having aFlu greater than an arbitrary channel number or the channel number that corresponds to an arbitrary percent of cells. In general, the criterion chosen to characterize aFlu increases was the percentage of cells with high 90°Sc (usually > channel 55) that registered aFlu > channel 100.

Fifteen experiments comparing 37°C incubations with or without HPCM were carried out. In all experiments, HPCM increased aFlu (Fig. 3). The aFlu profiles of cells incubated at 4°C and at 37°C with HPCM were similar, raising the possibility that HPCM may act by preventing the decay of aFlu of eosinophils. However, this cannot be the entire explanation of the action of HPCM, since it stimulated aFlu above that seen at 4°C (71%, 50%, and 61% cells with aFlu > channel 100 in 37°C incubations with or without HPCM and 4°C incubations, respectively, and see Fig. 6).

In control experiments, HPCM heated for 1 hr at 56°C did not lose activity, and possible contaminants of HPCM, for example, lipopolysaccharide (LPS), or substances in normal human serum failed to augment eosinophil aFlu (data not shown). Increases in aFlu were detectable after 1-hr incubation and increased thereafter (percent of cells with aFlu > channel 100 at 0, 1, 2, and 3 hr were 32%, 39%, 58%, and 62%, respectively). It was also noted that serum did not inhibit the activity of HPCM (the percent cells with aFlu > channel 100 with and without HPCM was 66% and 40%, and with 5 sera at 10% final concentration, 64.4% ± 3.5% and 36.8% ± 6.1%, respectively). Finally, although the purity of cell preparations was not 100%, the 1%-10% contaminating neutrophils are unlikely to have altered the results as (1) they have a much lower aFlu than eosinophils (<10% have aFlu > channel 100 and <2% > channel 100 and <2% > channel 130 versus eosinophils with 85% and 65% aFlu cells in the same channel, respectively), and (2) the aFlu does not increase after incubation with CSF.
Density eosinophils showed a less intense aFlu than the higher density eosinophils, but showed similar increases after incubation with HPCM (Table 1).

**Effect of Purified Fractions of HPCM on Eosinophil aFlu**

Since HPCM contains many substances, it was of importance to identify the fraction responsible for increasing aFlu. Two different methods were employed. First HPCM was passed over an Ultrogel AcA44 column in order to separate it into proteins of different molecular weights. Fractions were then analyzed for protein concentration, granulocyte-macrophage (GM), and eosinophil (EO) CSA and capacity to increase eosinophil aFlu. The results (Fig. 4) show that GM and EO-CSA cochromatographed with the capacity to increase the aFlu of mature eosinophils and showed an apparent molecular weight of 30,000. Active fractions were tested on 3 occasions, and the mean percentage of cells with aFlu >100 incubated with active fractions was 54.4% ± 1.5% whereas those incubated without stimulation was 28.8% ± 9.0% (p < 0.05).

Secondly, active fractions from AcA44 columns were pooled and passed over phenyl Sepharose columns. The unbound (CSF-a) and the bound fraction (CSF-β) were collected. Previously, Nicola et al. showed that, whereas both CSF-α and CSF-β possessed GM-CSA, only CSF-α possessed EO-CSA. Similar to the CSA data, only CSF-α, and not CSF-β, increased the aFlu of mature eosinophils (Fig. 5).

This experiment was repeated 5 times with cells from different individuals. The mean percent of cells with aFlu > channel 100 incubated with CSF-α was 64.6% ± 2.7% and with CSF-β was 39.6% ± 6.4% (arithmetic mean ± SEM). These values differ at the p < 0.01 level. These studies suggest that a molecule similar to human CSF-α is responsible for effects on mature human eosinophils and will henceforth be referred to as CSF.

**Table 1. The Effect of HPCM on aFlu of Eosinophils of Varying Density**

<table>
<thead>
<tr>
<th>Metrizamide Fraction</th>
<th>Conditioned Medium</th>
<th>Percent Eosinophils With aFlu Greater Than Channel</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p Value</td>
</tr>
<tr>
<td>23</td>
<td>+</td>
<td>43.9 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>34.1 ± 2.1</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>62.1 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>48.1 ± 1.1</td>
</tr>
</tbody>
</table>

*Percentage of metrizamide gradient from the top of which cells are recovered.
†HPCM or CSF-α.
‡n = 5.
§n = 4.
||Arithmetic mean ± standard error.
Effect of Various Doses of CSF on Eosinophil aFlu

Purified eosinophils were incubated with serial dilutions of HPCM or CSF (AcA44 column-purified HPCM) for 18 hr at 37°C, washed, and analyzed on the FACS. The dose-response curves in Fig. 6 show that the increase in aFlu was dose-dependent in both cases. Cells maintained at 4°C (with or without CSF) had aFlu that corresponded to intermediate doses of CSF. HPCM was only tested from a dilution of 1/160, since this unpurified form of CSF is known to contain inhibitors of CSA at lower dilutions. In both cases, dose-response curves for colony-formation and increase in eosinophil aFlu were similar.

Effect of Mouse CSF Containing Substances Influencing Human Eosinophil aFlu

Mouse lung and mouse spleen conditioned media (MLCM and MSCM) contain mouse CSF and were tested for their effect on mature human eosinophils. MSCM increased eosinophil aFlu in a dose-dependent fashion. In contrast, MLCM had no effect (Table 2). Both substances were used in concentrations that had maximal colony-stimulating activity on mouse bone marrow. Interestingly, mouse MSCM, but not MLCM, contains eosinophil CSA, while both contain GM-CSA (Metcalf, Cutler and Nicola, submitted for publication).

Effect of CSF on New Protein Synthesis by Eosinophils

Eosinophils incubated with or without CSF (AcA44 fraction) in methionine-free medium with [35S]-methionine were analyzed on the FACS and sorted into cells with increasing degrees of aFlu. The incorporation of [35S]-methionine into cells is shown in Fig. 7 and indicates that cells with higher aFlu took up more label. Next, the effect of CSF on the uptake of label was investigated. Table 3 shows that CSF increased the amount of label incorporated into cells and that this label was TCA precipitable.
Effect of CSF on Superoxide Anion Production by Eosinophils

Purified eosinophils at 10^5/ml were incubated with CSF-α, β, or medium for 10 min at 37°C and then cytochrome-c was added alone or with particulate (preopsonized zymosan) or soluble (PMA) stimulus. The reactions were terminated by rapid chilling. The results (Table 4) show that CSF-α, but not β, stimulated the reduction of cytochrome-c.

Effect of CSF on Iodination by Eosinophils

Purified eosinophils (10^6/ml) were incubated with or without a stimulus in the presence on absence of CSF-α or CSF-β and ^125^I. The reactions were terminated 60 min after the addition of CSF by cold TCA. The results (Table 5) show that CSF-α, but not β, stimulated iodination by eosinophils, and that, in contrast to superoxide production, the effects of CSF-α and PMA were additive.

**DISCUSSION**

Eosinophils are specialized cells that contain a unique toxic protein (eosinophil major basic protein, E-MBP) that can destroy parasites as well as damage normal human tissues. Populations of eosinophils that have an enhanced capacity to destroy parasites have been found in eosinophilic individuals. Subsequently, eosinophils from eosinophilic patients were also found to differ from normal eosinophils in their surface charge, uptake of _3^H-2-deoxyglucose, lysozomal acid phosphatase, and response to zymogen-activated serum. Eosinophils with increased capacity to kill parasites have also been produced in vitro after incubation with certain T-cell products, ECF-A, and, most powerfully, with HPCM. These "activated" eosinophils seem to have, on the one hand, the desirable property of enhanced helminthotoxicity, but on the other hand, the increased potential to damage normal tissues, and hence, produce disease. The process that leads to or prevents activation is thus an important one to understand.

Eosinophil function can be measured by a variety of methods, including superoxide production and iodination. Incubation with HPCM powerfully activated superoxide production and iodination (Tables 4 and 5). The increase was additive with another stimulus, preopsonized zymosan, and in the case of iodination, also with PMA, a substance that is often thought to give maximal stimulation. Thus, a case can be made for HPCM-derived molecules as a powerful class of molecules controlling eosinophil function.

In addition, we developed a method of analysis of the interaction of eosinophils and HPCM by the FACS. The measurement of aFlu of a population of eosinophils appears to reflect the degree of activation, since only those fractions that stimulate O_2^- production...
Table 4. Effect of CSFs on Eosinophil-Mediated Superoxide Production

<table>
<thead>
<tr>
<th>Superoxide Production (nmole Cytochrome-c Reduced/10^5 Eosinophils/hr)</th>
<th>p Value of Difference Between Values With CSF-α and Nil or CSF-β</th>
</tr>
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<tbody>
<tr>
<td>NIL</td>
<td>Preincubation With CSF-α</td>
</tr>
<tr>
<td>NIL</td>
<td>0.03 ± 0.03†</td>
</tr>
<tr>
<td>Preopsonized zymosan</td>
<td>0.15 ± 0.06</td>
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<tr>
<td>PMA</td>
<td>2.04 ± 0.57</td>
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*The addition of superoxide dismutase completely inhibited the reduction of cytochrome-c.
†Arithmetic mean ± SD of 3 experiments performed in duplicate.

Table 5. Effect of CSF on Eosinophil-Mediated Iodination

<table>
<thead>
<tr>
<th>Iodination (nmole 125I Incorporated/10^5 Eosinophils/hr)</th>
<th>p Value of Difference Between Values With CSF-α and Nil or CSF-β</th>
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</thead>
<tbody>
<tr>
<td>NIL</td>
<td>Preincubation With CSF-α</td>
</tr>
<tr>
<td>NIL</td>
<td>0.76 ± 0.16*</td>
</tr>
<tr>
<td>Preopsonized zymosan</td>
<td>7.52 ± 2.08</td>
</tr>
<tr>
<td>PMA</td>
<td>10.72 ± 2.16</td>
</tr>
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</table>

*Arithmetic mean ± SD of 2 experiments performed in duplicate.

The material in HPCM responsible for eosinophil activation appears to be similar to CSF. The two activities cochromatograph on an Ultrogel-AcA44 column (Fig. 4) and have similar dose-response characteristics (Fig. 6). However, it is only a subspecies of human CSF, CSF-α, that can activate mature human eosinophils.

CSF-α can be separated from another subspecies of CSF, CSF-β, on phenyl Sepharose chromatography. CSF-α is the less hydrophobic moiety and contains both EO-CSA and GM CSA; it preferentially stimulates day-14 colonies and acts primarily on progenitor cells that bind little fucose-binding protein and sediment at 1 g at a velocity of 4–6 mm/hr (Johnson, G. R. and Nicola, N. A., personal communications). In contrast, the more hydrophobic moiety of CSF, CSF-β, has only GM-CSF activity, preferentially stimulates day-7 colonies, and acts on progenitor cells that bind more fucose-binding protein and sediment at a velocity of 7–9 mm/hr. These experiments suggest that CSF-α and CSF-β act on different populations of progenitor cells. It is, however, still possible that these populations are different maturational stages of the same lineage; CSF-α acting on the less differentiated cell. Our observations that CSF-α, but not CSF-β, stimulates circulating eosinophils strongly support the notion that EO-CSF has an action on mature eosinophils and suggest that a receptor of similar specificity is involved in the stimulation of progenitor cells and the differentiated progeny of these cells.

The capacity of MSCM, but not MLCM, to activate mature human eosinophils also supports this notion.
since MSCM, but not MLCM, stimulates the production of eosinophil colonies from mouse bone marrow.\textsuperscript{18,19} Indeed, it has recently been shown that MSCM, but not MLCM, can stimulate human eosinophil colony formation (Metcalfe, D., Cutler, R., Nicola, N., submitted for publication). The stimulatory activity of regulators of hemopoiesis on mature cells has been previously demonstrated in the murine system.\textsuperscript{25-28} However, to our knowledge, this is the first demonstration that only a subspecies of human CSF has such a stimulatory action.

The measurement of eosinophil activation on the FACS depends on the degree of aFlu of eosinophils. Although high eosinophil aFlu has been described previously in both man\textsuperscript{29} and mouse,\textsuperscript{30} there is no information regarding the mechanism of this phenomenon. Both we (Watt and Vadas, in preparation) and others\textsuperscript{31,32} have observed that the eosinophil is the most aFlu of all blood cells, and it is tempting to suggest that the most characteristic component of eosinophils, the eosinophil granule, is responsible. Indeed, we observed that eosinophil granules do have a degree of aFlu. However, since neutrophils are also autofluorescent, the aFlu of eosinophil granules is unlikely to provide the full explanation.

Finally, it must be emphasized that there are likely to exist several pathways that lead to eosinophil activation as measured by increased capacity to destroy parasites. Some stimuli, like ECF-A, may act primarily by increasing the expression of surface receptors that allow a greater degree of adherence to the organisms.\textsuperscript{33} CSF-like molecules may act primarily by increasing the metabolic activity of the cell. Interestingly, ECF-A does not increase eosinophil aFlu or protein synthesis (Varigos and Vadas, in preparation), pointing to a mechanism of action different from CSF. This finding therefore raises the possibility of synergy between the stimuli, and this is under investigation.

Thus the circulating eosinophil can no longer be thought of as a dormant mature cell. It is sensitive to a number of stimuli that regulate its prowess as an effector and, perhaps, as an autotoxic cell.

\textbf{ACKNOWLEDGMENT}

We would like to acknowledge the excellent technical assistance of Lucy Callegaro. We thank Dr. I. Mackay for his encouragement and for reading the manuscript.

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Eosinophil activation by colony-stimulating factor in man: metabolic effects and analysis by flow cytometry

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