Human Eosinophils Have Prolonged Survival, Enhanced Functional Properties, and Become Hypodense When Exposed to Human Interleukin 3

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Abstract

Human eosinophils were cultured in the presence of recombinant human IL-3 for up to 14 d and their biochemical, functional, and density properties were assessed. After 3 d of culture in 10 pM IL-3, eosinophils had a viability of 70% compared with only 10% in enriched medium alone. Neither IL-1α, IL-2, IL-4, tumor necrosis factor, basic fibroblast growth factor, nor platelet-derived growth factor maintained eosinophil viability. The 7- and 14-d survival of the cultured eosinophils was 55 and 53%, respectively. No other cell type, including neutrophils, was present after culture. After 7 d of culture, the normodense eosinophils were converted to hypodense cells as assessed by density centrifugation. Eosinophils exposed to 1,000 pM IL-3 for 30 min or cultured in 10 pM IL-3 for 7 d generated approximately threefold more leukotriene C4 (LTC4) in response to calcium ionophore than freshly isolated cells. Furthermore, whereas freshly isolated eosinophils killed only 14% of the antibody-coated Schistosoma mansoni larvae, these eosinophils killed 54% of the larvae when exposed to 100 pM IL-3. The enhanced helminth cytotoxicity was maintained for 7 d when eosinophils were cultured in the presence of both 10 pM IL-3 and 3T3 fibroblasts, but not when eosinophils were cultured in the presence of IL-3 alone. IL-3 thus maintains the viability of eosinophils in vitro, augments the calcium ionophore-induced generation of LTC4, enhances cytotoxicity against antibody-sensitized helminths, and induces the eosinophils to become hypodense cells. These phenotypic changes in the eosinophil may be advantageous to host defense against helminthic infections but may be disadvantageous in conditions such as allergic disease.

Introduction

The viability of human peripheral blood eosinophils can be maintained in vitro for at least 14 d when these cells are cocultured in the presence of either human or bovine endothelial cells (1) or a combination of human recombinant granulocyte/macrophage–colony-stimulating factor (GM-CSF)1 and mouse 3T3 fibroblasts (2). As compared with replicate freshly isolated normodense eosinophils, the cultured eosinophils increased their capacity to generate leukotriene C4 (LTC4) in response to calcium ionophore, increase their cytotoxicity against antibody-coated Schistosoma mansoni larvae and are converted to hypodense cells as assessed by sedimentation analysis. Hypodense eosinophils, defined by their lower than normal sedimentation density in discontinuous metrizamide or Percoll gradients, have been found in the peripheral blood of some patients with eosinophilia-associated diseases such as the idiopathic hypereosinophilic syndrome and chronic infection with S. mansoni (3-6). These eosinophils generate more LTC4 in response to activation with calcium ionophore (7) and kill more antibody-coated S. mansoni larvae (5) than normodense eosinophils.

IL-3 and GM-CSF stimulate bone marrow progenitors in vitro to form colonies of cells that include eosinophils (8-10). However, IL-3 is distinct from GM-CSF because it induces bone marrow progenitors to form colonies that are more diverse in cell type. In addition, murine IL-3 preferentially stimulates the growth and differentiation of mast cells in culture (11). Gibbon recombinant IL-3 augments the functional properties of mature human peripheral blood eosinophils by acutely increasing their generation of superoxide, their phagocytosis of opsonized yeast, and their antibody-dependent killing of tumor cells (10).

In the present investigation, we report that human peripheral blood normodense eosinophils cultured with human recombinant IL-3 survive for at least 14 d and are converted to hypodense cells. Both acute and chronic exposure to IL-3 results in enhanced calcium ionophore–induced generation of LTC4. Acute treatment with IL-3 also increases eosinophil cytotoxicity against antibody-coated S. mansoni larvae; however, preserving this enhanced cytotoxic activity requires coculture of eosinophils in the presence of both IL-3 and 3T3 fibroblasts. These studies indicate that IL-3 and GM-CSF promote proinflammatory changes in eosinophils characterized by prolonged viability, enhanced functional properties, and hypodensity.

Methods

Isolation of human eosinophils. Human eosinophils were isolated by centrifugation in discontinuous metrizamide gradients (12, 13) from the peripheral blood of nine different donors, none of whom were ingesting corticosteroids, aspirin, or nonsteroidal antiinflammatory drugs. Two of these donors had no diagnosed clinical disorder and had normal white blood counts and differentials. The other seven donors were diagnosed as having allergic rhinitis, allergic conjunctivitis, and/or asthma; 2–10% of their white blood cells were eosinophils. Whole blood (45-ml aliquots) was aspirated into 60-ml syringes, each containing 1,000 U of sterile heparin (Sigma Chemical Co., St. Louis,
MO). All subsequent steps were performed under sterile conditions in a laminar flow hood using reagents that were filtered through 0.45-μm filters. To each syringe was added 9 ml of prewarmed PBS containing 4.5% (wt/vol) of 150,000–200,000 Mф, dextran (BDH Chemicals, Poole, England), pH 7.3. After a 45-min incubation at 37°C, the buffy coats were collected. An equal volume of isolation medium (RPMI-1640 supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, 10 μg/ml of gentamycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 10% [vol/vol] FCS, 1 mM Hepes [Gibco Laboratories, Grand Island, NY], and 45 μU of deoxyribonuclease-1 [Sigma Chemical Co., p'H 7.2]) was added and the cells were centrifuged at 250 g for 10 min at 4°C. The mixed leukocyte cell suspension was resuspended in isolation medium at a density of ∼ 3.0 × 10^6 cells/ml.

Metrizamide (Nyegaard and Co., Oslo, Norway) was dissolved in Tyrode's buffer containing 0.1% (wt/vol) gelatin and 45 μU of deoxyribonuclease to concentrations of 18, 20, 21, 22, 23, and 24% (wt/vol). The densities were standardized by their refractive indices (ρ = 1.3620, 1.3650, 1.3665, 1.3680, 1.3695, 1.3710 for the 18, 20, 21, 22, 23, and 24% metrizamide layers, respectively). Gradients were prepared by pipetting 2 ml of each solution of metrizamide stepwise into 15-ml tubes or by pipetting 7 ml of each solution into 50-ml tubes. After the addition of 1.5 ml of the leukocyte suspension into each 15-ml tube or 7 ml into each 50-ml tube, samples were centrifuged at 1,000 g for 45 min at room temperature. The cells recovered at the 22/23% and 23/24% gradient interfaces were pooled with those cells that pelleted at the bottom of the gradient. The purity of the starting population of metrizamide-purified normodense eosinophils was 84±10% (mean±SD, n = 22) as assessed by Wright's and Giemsa staining. Neutrophils were the principal leukocyte contaminant. Residual contaminating erythrocytes in the eosinophil preparations were eliminated by hypotonic lysis. Cell viability in all experiments was >98% as assessed by trypan blue (Gibco Laboratories) exclusion.

Culture of human eosinophils. Freshly isolated eosinophils (10^6) were resuspended in 180 μl of enriched medium (isolation medium without Hepes and without deoxyribonuclease) in 96-well, flat-bottom microtiter plates containing 20 μl of various dilutions of a specific cytokine. Cells were cultured for 3 d at 37°C in a humidified atmosphere of 5% CO_2. Human recombinant IL-3 was kindly provided by Dr. Y.-C. Yang (Genetics Institute, Cambridge, MA) and Dr. C. Sieff (Harvard School of Medicine, Boston, MA) as a Cos cell supernatant (14). The protein concentration of the Cos cell supernatant was ~1 μg/ml and consisted of ~90% IL-3. Purified human recombinant IL-3 that was expressed in Escherichia coli was also kindly provided by Dr. Y.-C. Yang. Purified human recombinant GM-CSF that was expressed in monkey Cos cells was kindly provided by Dr. Judith Gasson (University of California at Los Angeles, Los Angeles, CA) (15) and Dr. S. C. Clark (Genetics Institute) (16). Human recombinant IL-1α (Collaborative Research, Bedford, MA), human recombinant IL-2 (Cetus Corp., Emeryville, CA), human recombinant IL-4 (Dr. D. Rennick, DNAX, Palo Alto, CA), human platelet-derived growth factor, bovine pituitary basic fibroblast growth factor (Collaborative Research), and human recombinant tumor necrosis factor (Cetus Corp.) were also used in some experiments.

For 7–14-d culture experiments, freshly isolated eosinophils were resuspended at a density of 2.5–5.0 × 10^6 cells/ml in enriched medium containing 10 μM IL-3 or 10 μM GM-CSF. 2 ml of this suspension was seeded into 35-mm culture dishes containing or lacking a confluent monolayer of mouse 3T3 fibroblasts. Fibroblasts (line CCL 92; American Type Culture Collection, Rockville, MD) were grown in enriched medium as described previously (2). At 48-h intervals, the culture medium containing the suspension of eosinophils was aspirated, and 1 ml of fresh enriched medium was added directly to each culture plate. The eosinophils in each aspirate were centrifuged at 250 g for 10 min at room temperature, resuspended in 1.0 ml of fresh enriched medium containing IL-3 or GM-CSF, and added back to the appropriate original culture dish. All cultures were cultured under similar conditions except that IL-3 was either not added during the culture or was included only at the time of the initial seeding.

Analysis of the viability and density of eosinophils after culture. Eosinophils were recovered from each culture plate by washing the plate five times with 2 ml of enriched medium and pooling the collected suspension of cells. After centrifugation at 250 g for 10 min at 4°C, the cells were resuspended in 1 ml of enriched medium, and 50 μl was added to an equal volume of trypan blue. Those eosinophils that excluded trypan blue after a 5-min incubation at 37°C were considered viable cells. The number and percent of eosinophilic cells in the preparations were determined by counting the total cells with a hemacytometer and staining replicate samples of cytocentrifuge preparations with Wright's and Giemsa stains. The survival of the cultured eosinophils was assessed as follows. 15 µl of Eagles's modified medium containing 5% sera of cells excluding trypan blue/original number of eosinophils seeded. Color photomicrographs of eosinophils were taken through a Nikon Labaphore CFN microscope utilizing a Hitashi KPC-100 color video camera and printer (courtesy of the Monsanto Corp., Natick, MA). To assess the relative density of the eosinophils after culture, 0.5–2.0 × 10^6 cells were suspended in 1.5 ml of enriched medium and centrifuged over discontinuous gradients of metrizamide as described above. The eosinophils at each metrizamide interface were collected, diluted with ~10 ml of enriched medium, sedimented at 250 g for 10 min at 4°C, resuspended in 1 ml of enriched medium, and counted. Viability and morphology were assessed as described above.

Calcium ionophore-induced generation of LTB₄. Replicate samples of freshly isolated and cultured eosinophils were each incubated in 3 ml of modified Tyrode's buffer, pH 7.5, containing 0.3 mM KCl, 1 mM CaCl₂, 5 mM MgCl₂, 0.1% (wt/vol) gelatin, and 20 mM L-serine. The latter amino acid was added to prevent HOCl-dependent oxidative metabolism of LTB₄ (13). After two successive washes with modified Tyrode's buffer at 110 g for 10 min at 4°C, the replicate eosinophils were pooled and resuspended in the same buffer at a density of 2 × 10⁶ cells/ml. Samples (100 μl) of each cell suspension were centrifuged for 10 min at 37°C in a humidified atmosphere of 5% CO₂. The eosinophils that had been freshly isolated were then incubated with 100 μl of modified Tyrode's buffer lacking or containing IL-3 (0.04 to 4,000 PM) for 30 min at 37°C. As a positive control, eosinophils were also exposed to 20 μM GM-CSF for the same preincubation interval before the addition of the calcium ionophore. The cultured eosinophils were washed twice with modified Tyrode's buffer, and then samples (100 μl) of eosinophils at 2 × 10⁶ cells/ml were prewarmed for 10 min at 37°C in a humidified atmosphere of 5% CO₂. Calcium ionophore A23187 in 100 μl of buffer (final concentration 2.5-μM) (13) was added for 30 min at 37°C and the activation was terminated by adding 2 ml of methanol at 4°C. Each methanolic suspension was placed under argon at 4°C for ~14 h and then centrifuged at 500 g for 30 min at 4°C to remove proteins and cell debris. The methanolic extract was stored at −20°C under argon.

Immunoreactive LTB₄ was quantitated by RIA of the methanolic extracts of each reaction mixture. The methanolic extracts were evaporated to dryness under negative pressure (Speed Vac Concentrator; Savant Instruments, Hicksville, NY), and resuspended in Tris-Isogel buffer (0.1 M Tris-HCl, 0.14 M NaCl, and 0.1% gelatin, pH 7.4).

Sample (100 μl) were each mixed with 50 μl of H-labeled LTB₄(40 Ci/mmol; New England Nuclear, Boston, MA), and 100 μl of immune rabbit plasma as described (13, 17). Unbound H-labeled LTB₄ was removed by incubating samples for 15 min at 4°C with 1 ml of a 10:1 (wt/wt) suspension of charcoal/dextran T-40 (Sigma Chemical Co.) in Tris-Isogel buffer (2). The charcoal was removed by centrifugation at 2,000 g for 15 min at 4°C; the residual radioactivity in the supernatant was quantified by β-scintillation counting. In this RIA, synthetic LTB₄ was detectable on the linear portion of the radioligand inhibition-binding curve at concentrations ranging from 0.1 to 1.0 ng. Statistical analysis was determined by the two-tailed t test.

Cytotoxicity assay. Freshly isolated and cultured eosinophils were washed twice with 15 ml of Eagle's modified essential medium containing 100 U/ml of penicillin, 100 μg/ml of streptomycin, 2 mM L-glutamine, 1 mM Hepes, and 10% heat-inactivated (56°C, 30 min)
fetal bovine serum (enriched MEM). Cells were resuspended at a density of 2 × 10^6 cells/ml in enriched MEM, and 50-μl samples were added to individual wells of a 96-well, round-bottomed microtiter plate. 50 μl of enriched MEM lacking cytokine, or containing either GM-CSF (40 pM) or IL-3 (0.04 to 4,000 pM) was added, and the eosinophils were incubated for 15 min at 37°C in a sealed, humidified box. A 50-μl portion of enriched MEM containing pooled, heat-inactivated sera (1:50 final dilution) from patients with S. mansoni infections, along with 50 μl of mechanically transformed S. mansoni larvae (2 × 10^7 worms/ml) were added to each well (18). The viability of the larvae was assessed 24 h later by microscopy using motility, contractility, and toluidine blue exclusion as criteria (19). In some wells, eosinophils were omitted to ensure that none of the reagents directly influenced the viability of the schistosomula.

Results

IL-3 enhancement of eosinophil viability. As assessed by their uptake of trypan blue, only 10±6% (mean±SD, n = 10) of the starting population of human eosinophils were viable after 3 d of culture in enriched medium alone (Fig. 1). In contrast, replicate samples of eosinophils cultured in enriched medium supplemented with incremental concentrations of IL-3 showed a dose-dependent enhanced viability at 3 d. A maximum viability of 70±7% (mean±SD, n = 10) was obtained at a 10 pM concentration of IL-3 (Fig. 1), while the concentration of IL-3 that resulted in a 50% maximal viability was 0.1 pM. Using purified IL-3, eosinophils from two different donors had a maximal 3-d viability of 80 and 78% at 10 and 100 pM IL-3, respectively. When eosinophils were cultured for 7 d (n = 8) and 14 d (n = 3) in the presence of 10 pM IL-3 under conditions in which the medium was replaced every 48 h with fresh IL-3 containing medium, 55±12 (mean±SD) and 53±21% (mean±SD), respectively, of the starting eosinophils remained viable (Fig. 2). In contrast, no eosinophils survived to 4 d in enriched medium alone (n = 4), and only 5±4% (mean±range, n = 2) survived to 7 d when exposed to IL-3 for only the first 48 h of the culture (Fig. 2). Fresh eosinophils (Fig. 3 A) and IL-3 treated eosinophils (Fig. 3 B) both contained eosin-staining granules and segmented nuclei as assessed by Wright's and Giemsa stains of cytocentrifuge preparations.

The effect of IL-3 on eosinophil viability appeared to be specific since neutrophils, the contaminating cell in the initial population of seeded leukocytes, were not present by day 2 of culture in 10 pM IL-3. In a 3-d assay of other cytokines, IL-1α (10^-3 to 10^-2 U/ml), tumor necrosis factor (10^-1 to 10^-3 U/ml), IL-2 (1 to 10^3 U/ml), IL-4 (10^-8 to 10^-2 dilution), platelet-derived growth factor (10^-1 to 10 U/ml), and basic fibroblast growth factor (10^-1 to 10^2 ng/ml) were each unable to maintain the viability of eosinophils above the baseline achieved in enriched medium alone.

In two experiments, eosinophils were cultured for 3 d in the presence of incremental concentrations of either IL-3 (0.01 to 100 pM) or GM-CSF (0.1 to 50 pM) alone and in combination with a fixed suboptimal concentration of the other cytokine, 1.0 pM GM-CSF or 0.1 pM IL-3. Culture of eosinophils in enriched medium containing both cytokines did not improve their viability compared with culture in medium containing either cytokine alone (Fig. 4). In two experiments, eosinophils cultured for 7 d in the presence of optimal concentrations of both 10 pM IL-3 and 10 pM GM-CSF, had a survival of 54±12% (mean±range, n = 2) as compared with 49±2% for 10 pM IL-3 alone.

Sedimentation density of eosinophils cultured in IL-3. The freshly isolated eosinophils that were cultured in 10 pM IL-3 were routinely selected from the normodense fractions 5, 6, and 7 of the discontinuous metrizamide gradients. When the density of the cultured eosinophils was assessed after either 3 or 7 d of culture, 73±16 and 93±5% (mean±SD, n = 3) of the eosinophils, respectively, were recovered in the hypodense gradient fractions 1, 2, and 3. The results from a representative experiment are shown in Fig. 5.

IL-3 augmentation of calcium ionophore-induced generation of LTC4 by eosinophils. When eosinophils were cultured in enriched medium supplemented with 10 pM IL-3 for 7 d, washed and activated with 2.5 μM calcium ionophore in modified Tyrode's buffer, they generated approximately threefold more LTC4 (P < 0.05) than freshly isolated cells (Table I).

The effect of exposure to enriched medium alone could only be assessed over a limited time interval because of a loss of viability. Whereas eosinophils preincubated for 60 min at 37°C in modified Tyrode's buffer generated 27±8 ng of LTC4/10^6 cells (mean±SD, n = 3), replicate eosinophils incubated in enriched medium generated 79±32 ng upon activation with calcium ionophore. In order to further assess the ability of short-term exposure to IL-3 to also increase LTC4 production, eosinophils were exposed to IL-3 in the presence of modified Tyrode's buffer. In two experiments in which eosinophils were incubated for 2, 5, 10, 30, 60, and 90 min with 10 or 100 pM IL-3, a plateau for enhanced LTC4 generation occurred at 30 min. Whereas preincubation in modified Tyrode's buffer alone did not increase LTC4 generation, there was a dose-dependent increase in the calcium ionophore-induced LTC4 generation from eosinophils that had been ex-
posed to increasing concentrations of IL-3 from 100 to 1,000 pM without a plateau (Fig. 6). Exposure of eosinophils to 100 and 1,000 pM IL-3 increased LTC4 generation from 22±4 to 38±9 and to 62±3 ng/10^6 cells (mean±SD, n = 3), respectively (P < 0.01 for both doses of IL-3).

**IL-3 enhancement of eosinophil cytotoxicity against antibody-coated *S. mansoni* larvae.** As shown in Fig. 7, when eosinophils were exposed to increments of IL-3, there was a dose-dependent increase in their cytotoxicity against antibody-coated *S. mansoni* larvae. Whereas freshly isolated eosinophils killed only 14±6% (mean±SD, n = 4) of these helminth targets, in the presence of 10 pM or 100 pM IL-3, replicate eosinophils killed 42±22 or 54±16% (mean±SD), respectively, of the targets (P < 0.05 and < 0.02, respectively). Eosinophils cultured for 7 d in the presence of concentrations of IL-3 and GM-CSF that were optimal for maintenance of viability did not have enhanced cytotoxicity compared with replicate freshly isolated eosinophils (Table II). Eosinophils had enhanced cytotoxicity (P < 0.05) against the larvae, as compared with freshly isolated untreated eosinophils, only if they were cocultured with 3T3 fibroblasts in the presence of IL-3 or GM-CSF (Table II). When freshly isolated eosinophils were cocultured with 3T3 fibroblasts for up to 2 h before their incubation with antibody-coated *S. mansoni* larvae there was no enhancement of eosinophil cytotoxicity. In other experiments, the addition of 48-h fibroblast-conditioned medium at a concentration of 1–25% had no effect on the killing assay.

**Discussion**

Human recombinant IL-3, like human recombinant GM-CSF (2), maintains the viability of human peripheral blood eosino-
Eosinophils for at least 14 d in vitro and enhances the functional properties of these cells. The viability of eosinophils after 3 d of culture was improved from a mean of 10% in enriched medium alone to a mean of 70% in the presence of 10 pM IL-3 (Fig. 1). Eosinophils cultured for 3 d in the presence of various concentrations of IL-3 and GM-CSF did not have an enhanced viability compared with an optimal concentration of either cytokine alone (Fig. 4). Under conditions in which the IL-3 supplemented enriched medium was replaced every 48 h, eosinophil survival was constant by day 4 of culture, was a mean of ~55% by day 7, and was maintained for at least seven

Figure 4. The effect of a fixed suboptimal concentration of 0.1 pM IL-3 or 1.0 pM GM-CSF on the dose-dependent 3-d viability of eosinophils in response to the other cytokine. (A) Eosinophils were cultured with incremental concentrations of IL-3 in the absence (●) or presence (○) of 1.0 pM GM-CSF. (B) Eosinophils were cultured with incremental concentrations of GM-CSF in the absence (●) or presence (○) of 0.1 pM IL-3. The results are the mean viability of two experiments done in duplicate.

Figure 5. The density gradient distribution of (A) freshly isolated eosinophils and eosinophils cultured in the presence of 10 pM IL-3 for (B) 3 or (C) 7 d. Discontinuous metrizamide gradients fractions 1-6 refer to the eosinophils recovered at the 0/18, 18/20, 20/21, 21/22, 22/23, and 23/24% interfaces, respectively; fraction 7 refers to the eosinophils in the cell pellet. The open bars and the stippled bars refer to eosinophils sedimenting in the normodense fractions (5, 6, and 7) and the hypodense fractions (1, 2, and 3), respectively. The results are representative of three experiments for which the 7-d survival was 56±3% (mean±SD).

Table I. LTC4 Generation by Calcium Ionophore-activated Eosinophils before and after 7 d of Culture in 10 pM IL-3

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<th>Exp.</th>
<th>Fresh eosinophils</th>
<th>Eosinophils cultured in IL-3</th>
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<td>1</td>
<td>17</td>
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<td>5</td>
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<td>Mean±SD</td>
<td>38±36</td>
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*The statistical significance between cultured and freshly isolated eosinophils was determined by the paired two-tailed t test.*

The data are expressed as nanograms of LTC4/10^6 cells.

additional days (Fig. 2). The effect of IL-3 on eosinophils appeared to be cell specific since neutrophils, the contaminating cell in the initially seeded leukocytes, did not survive after 2 d of culture. Eosinophils cultured in various concentrations of IL-1α, IL-2, IL-4, tumor necrosis factor, basic fibroblast growth factor, or platelet-derived growth factor did not have prolonged viability, indicating that the effect of IL-3 and GM-CSF on eosinophil viability was relatively cytokine specific.

Eosinophils cultured for 7 d in the presence of enriched medium supplemented with 10 pM IL-3, washed, and activated in modified Tyrode’s buffer, generated approximately threefold more LTC4 in response to 2.5 μM calcium ionophore A23187 as compared with freshly isolated eosinophils (Table I). Freshly isolated eosinophils, acutely exposed to either enriched medium alone or to 1,000 pM IL-3 in modified Tyrode’s buffer (Fig. 6) also generated a threefold increased amount of LTC4 in response to calcium ionophore as compared with cells exposed to Tyrode’s buffer alone before activation. These findings suggested that the isolation and purification procedure may attenuate the 5-lipooxygenase pathway and that IL-3 or GM-CSF (2, 19) induces the cells to restore their usual capacity to generate LTC4. Nevertheless, the calcium ionophore-induced generation of large amounts of LTC4 by the cultured cells indicates that this arachidonic acid pathway is maintained during culture.

The antibody-dependent cytotoxicity of freshly isolated eosinophils against S. mansoni larvae was increased threefold and fourfold in the presence of 10 and 100 pM IL-3, respectively (Fig. 7). Surprisingly, eosinophils cultured for 7 d in the

Figure 6. The acute effect of IL-3 on the dose-dependent calcium ionophore A23187-induced generation of LTC4 by freshly isolated eosinophils. The results are expressed as the mean±SD for three experiments. As a positive control, replicate eosinophils acutely exposed to 10 pM GM-CSF generated 56±23 ng LTC4/10^6 cells.
The presence of either 10 pM IL-3 or 10 pM GM-CSF did not have enhanced cytotoxicity compared with replicate freshly isolated eosinophils (Table II) even though comparable cell preparations generated large amounts of LTC4 and were of the hypodense phenotype. Although we had observed that eosinophils cultured for 7 d in GM-CSF in the presence of 3T3 fibroblasts had enhanced cytotoxicity against antibody-coated S. mansoni larvae compared with freshly isolated cells, we had not studied the cytotoxicity of eosinophils maintained by GM-CSF in the absence of 3T3 fibroblasts (2). When eosinophils were cultured in enriched medium containing either 10 pM IL-3 or 10 pM GM-CSF and in the presence of 3T3 fibroblasts, they had enhanced cytotoxicity against antibody-coated S. mansoni larvae compared with freshly isolated eosinophils or eosinophils maintained by cytokines alone. Coculture of freshly isolated eosinophils with 3T3 fibroblasts for up to 2 h (or the addition of 3T3 conditioned medium to the killing assay) did not enhance eosinophil cytotoxicity. These findings suggested that there was no short-term direct effect of the 3T3 fibroblasts on the cytotoxic potential of freshly isolated eosinophils. The long-term IL-3 independent effects of 3T3 fibroblasts on eosinophil cytotoxicity could not be assessed because 3T3 fibroblasts alone do not maintain the viability of eosinophils (1, 2). 3T3 fibroblasts have been shown to regulate the viability and functional properties of rodent (20, 21) and human (22) mast cells, and thus a connective tissue microenvironment appears to regulate certain properties of both eosinophils and mast cells.

During the first 7 d of culture in 10 pM IL-3, eosinophils were progressively converted from normodense to hypodense (Fig. 5). Hypodense eosinophils have been found in the peripheral blood of patients with chronic helminthic infections (3), bronchial asthma (5, 23), atopy (4), the idiopathic hypereosinophilic syndrome (3–6), and neoplasia (6). Hypodense eosinophils have enhanced antibody-mediated cytotoxicity (5), enhanced ligand-initiated chemotactic activity (24), enhanced ionophore-induced generation of LTC4 (7), increased consumption of oxygen and glucose (4–6), and enhanced expression of IgE low affinity receptors (25) compared with normodense eosinophils. The ability to convert normodense eosinophils into hypodense cells by culture with IL-3 suggests that IL-3 may be one of the cytokines that regulates the survival and phenotypic properties of eosinophils in these various disease states. That normodense eosinophils are converted into hypodense cells by culture in IL-3 or GM-CSF (2), or by coculture with human or bovine endothelial cells (1), suggests that the hypodense state may be the phenotype of mature eosinophils in inflamed tissue where they may be chronically exposed to cytokines produced by T cells or connective tissue cells. This possibility is supported by the finding of hypodense eosinophils in the pleural exudate of some patients with peripheral blood eosinophilia (4) and pulmonary infiltrative eosinophilia (6), even though the eosinophils present in their blood are predominantly normodense.

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