Differentiation In Vitro of Hybrid Eosinophil/Basophil Granulocytes: Autocrine Function of an Eosinophil Developmental Intermediate

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Summary

Granulocytes with the hybrid characteristics of eosinophils and basophils have been identified in the bone marrow and peripheral blood of humans with myeloid leukemias. We now describe a technique by which such hybrid granulocytes can be developed in vitro from normal cord blood precursors cultured in the presence of recombinant human interleukin (rhIL) 3 (350 pM) and rhIL-5 (200 pM) in a plastic vessel coated with Matrigel™. After 14 d in culture, 90 + 3% (mean ± standard error of the mean) of the nonadherent cells cultured in the Matrigel-coated flasks contained both eosinophil and basophil granules, as indicated by staining with Wright's and Giemsa stains. Of the nonadherent cells, 93 ± 1% contained cyanide-resistant peroxidase, and 88 ± 2% were toluidine blue-positive, characteristic of eosinophil and basophil granules, respectively. Transmission electron micrographs showed hybrid cells containing ultrastructurally distinct eosinophil granules with developing crystalline cores and basophil granules with reticular structures. These 14-d cord blood-derived cell cultures showed strong hybridization signals for eosinophil-derived neurotoxin by RNA blot analysis and contained 78 ng histamine per 10⁶ cells. When the granulocytes were removed from cytokine-containing medium and suspended without Matrigel in RPMI 1640 medium containing 10% fetal calf serum (FCS), more than 80% of the granulocytes excluded trypan blue for as long as 5 d, and 93% had developed into eosinophils at 6 d. Conditioned medium prepared over 48 h from the 14-d cell cultures (hybrid granulocytes) sustained the 4-d viability in vitro of 78% of peripheral blood eosinophils from atopic donors. In comparison, 13% survived in RPMI 1640 containing 10% FCS alone. This viability-sustaining activity was nearly completely neutralized by an anti-granulocyte/macrophage colony-stimulating factor (GM-CSF) antibody and was only minimally reduced by anti-IL-3 or -IL-5. Thus, cells possessing both eosinophil and basophil granules by both histochemical and ultrastructural analysis can be developed from normal progenitors in vitro in response to eosinophilopoietic cytokines and Matrigel. Their subsequent spontaneous development into mature eosinophils suggests that hybrid granulocytes are part of a normal developmental sequence during eosinophilopoiesis. Furthermore, these hybrid granulocytes are capable of autoregulation through elaboration of GM-CSF, which sustains their viability.

Eosinophils and basophils have many common characteristics, such as granules that contain major basic protein (1) and lysophospholipase (2), a 5-lipoxygenase pathway (3–5), and receptors for IL-3, IL-5, and GM-CSF (6, 7). Patients with basophilia often have eosinophilia (8), and a rare syndrome has been described characterized solely by the absence of both cell types (9). Thus, although differences exist between mature basophils and eosinophils, such as the presence of histamine in basophil granules (10), granule ultrastructure (11), and staining properties of the granules and nuclear morphology (8), evidence supports a common lineage.

It has been suggested that eosinophils and basophils arise from a common eosinophil/basophil lineage intermediate that has coexistent eosinophil and basophil granules ("hybrid" granulocytes) (10, 12). Such hybrid granulocytes have been identified in the peripheral blood of patients with chronic myelogenous leukemia (12, 13) and in the marrow of patients with M4 type acute myelogenous leukemia (14, 15). Furthermore, when peripheral blood leukocytes from M4-type acute myelogenous leukemia patients were transplanted into mice with severe combined immunodeficiency that were then treated with intraperitoneal c-kit ligand and a fusion cytokine...
containing both IL-3 and GM-CSF, leukocyte engraftment resulted and cells that resembled eosinophils with prominent basophilic granules appeared in both the marrow and the peripheral circulation (16). Based on the criteria of cell size and nuclear morphology, hybrid granulocytes have been characterized as abnormal eosinophils having basophil granules (14–16) or basophils having eosinophil granules (12, 13). These cells exhibit metachromatic staining with toluidine blue and contain a cyanide-resistant peroxidase characteristic of eosinophils (12). Additional experimental support for a hybrid eosinophil/basophil progenitor includes the observation of leukocytes with both eosinophil and basophil granules in hematopoietic colonies derived from normal peripheral blood progenitors grown in semisolid methylcellulose medium supplemented with conditioned medium from a hairy cell leukemia cell line, Mo (10). These findings suggest that such “ambiguous” cells might be part of a normal developmental sequence that is more readily apparent in patients with certain hematologic malignancies.

Various techniques have been described for the development of eosinophils and basophils in vitro from peripheral blood, cord blood, and bone marrow progenitors (10, 11, 17–19). When cord blood–derived precursors were cultured in the presence of recombinant human (rh) IL-3, the cell population contained 39% eosinophils and 22% basophils after 14 d and 81% eosinophils after 21 d (17). Cord blood progenitors maintained in culture for 21–35 d in the presence of both rhIL-3 and rhIL-5 developed into populations of 80–95% eosinophils, with basophils among the remaining cells (20). Only the study of Denburg et al. (10), which used an uncharacterized conditioned medium to develop eosinophil and basophil colonies, found that 5–48% of the cells possessed both granule types.

We now describe the development of ∼90% pure eosinophil/basophil hybrid granulocytes from human umbilical cord blood mononuclear cells cultured with the cytokines rhIL-3 and rhIL-5 along with a soluble extracellular matrix (Matrigel™). The inclusion of the latter appears to accelerate the acquisition of granules and supports the development of the hybrid phenotype. It appears likely that this developmental intermediate is overrepresented in certain myelogenous leukemias (12–16). The fact that these hybrid granulocytes express a third eosinophilopoietic cytokine distinct from those used for their development represents a previously unrecognized autoregulatory capability in this lineage.

Materials and Methods

Culture System for Generating Granulocytes

Cord blood was drawn from the umbilical vein of the placenta after elective cesarean section deliveries under sterile conditions into 50-ml syringes containing 1,000 U heparin. The blood was layered onto a cushion of Ficoll-Paque™ (1.77 g/ml) in 50-ml sterile conical-bottom centrifuge tubes (1:1 vol/vol blood/Ficoll). After centrifugation of the tubes at 350 g for 30 min at room temperature, the mononuclear leukocytes at the interface were collected and pelleted (17). Contaminating erythrocytes were removed by hypotonic lysis with sterile 0.2% NaCl. The cells were counted in a hemocytometer, and cytospin slides were prepared and stained with Wright’s and Giemsa stains to provide an initial differential count. The mononuclear cells were suspended in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) containing 10% FCS, 50 μM β-mercaptoethanol, 200 μM rhIL-5 (R & D Systems, Minneapolis, MN), and 350 μM rhIL-3 (R & D Systems) at a density of 0.5–1.0 × 10⁶ cells per ml, and they were transferred with a pipette into culture flasks coated with 0.5 ml/cm² Matrigel (Collaborative Research, Inc., Bedford, MA) (21–24). As a control for the effects of Matrigel, duplicate cells were suspended in an uncoated flask. The culture medium containing the cytokines was changed every 7 d, at which time cell counts were obtained and cell viability was assessed by the exclusion of trypan blue.
Samples were postfixed with 1% OsO₄ in 0.1 M acetic acid buffer, pH 7.4, for 1 h at 4°C and enhanced with 0.5% uranyl acetate in 0.1% acetic acid buffer, pH 6.0, for 1 h at 37°C in the dark. The pellets were dehydrated and embedded in Epon at the optimal concentrations for the neutralization of 10 pM rhlb3, IL-5, and GM-CSF (Genzyme Corp., Cambridge, MA). A saturating concentration of antibody was added to a portion of total KNA. After the red blood cells were removed by repeated hypotonic lysis, the granulocyte pellets were incubated for 40 min at 4°C with immunomagnetic beads coupled to a rabbit anti-human CD16 antibody. The nonadherent eosinophil fraction was quantitated by counting in a hemocytometer. To assess purity, differential cell counts were performed after cytocentrifugation and staining with Wright's and Giemsa stains. Eosinophils were suspended at 106 cells per ml in RPMI 1640 with 10% FCS alone, in RPMI 1640 supplemented with 10 pM rhlb3, or in RPMI 1640 enriched with 50% conditioned medium (28, 29).

The identity of the eosinophil viability-sustaining activity found in the conditioned medium was sought with neutralization bioassays using monospecific polyclonal rabbit antibodies directed against human IL-3, IL-5, and GM-CSF (Genzyme Corp., Cambridge, MA). A saturating concentration of antibody was added to a portion of the wells in 96-well microtiter plates containing RPMI 1640 supplemented with 10% FCS and 25% of the 14-d in vitro-derived granulocyte-conditioned medium. After a 2-h preincubation, 10⁴ peripheral blood eosinophils were added to each well. Concentrations of antibody were chosen based upon a dose–response in which the optimal concentrations for the neutralization of 10 pM rhlb3, 10 pM rhlb5, and 10 pM rhGM-CSF were determined. For anti-IL-3 and anti-IL-5, optimal neutralization was achieved at concentrations of 100 µg/ml; for anti-GM-CSF, a concentration of 50 µg/ml was optimal. Cell viability was assessed by exclusion of trypan blue dye by 200 cells after 96 h in culture.

Statistical Analysis. The statistical significance of differences be-
Figure 1. Eosinophil/basophil granule development over 14 d as assessed by staining with Wright's and Giemsa stains of cells derived in vitro from cord blood mononuclear cells cultured in the presence of rhlL-3 and rhIL-5 with (white bars) and without (black bars) a thin layer of Matrigel. A cell was registered as granulated if it contained three or more eosinophilic or basophilic granules (n = 6, SEM noted in text).

Results

Histochemical, Biochemical, and Ultrastructural Analyses of Cord Blood-derived Granulocytes

Wright's and Giemsa Staining. After the cord blood mononuclear cells were cultured for 3 d with rhIL-3 and rhIL-5 in the presence or absence of Matrigel, <10% of the cells grown under either culture condition possessed eosinophil or basophil granules (Fig. 1). Over the next 11 d of culture, the cells developed in the presence of Matrigel acquired cytoplasmic granules in a far greater proportion than the cells developed in its absence. At 14 d of culture, 94 ± 3% (n = 6) of the cells grown on Matrigel had developed granules identifiable as basophilic (blue granules) or eosinophilic (red granules) by staining with Wright's and Giemsa stains; 90 ± 3% of the Matrigel-cultured cells contained both eosinophilic and basophilic granules (Fig. 2 A), and the remaining 4% with granules had either eosinophil or basophil granules only. In contrast, only 41 ± 3% of the cells grown on plastic had cytoplasmic granules (P <0.01 compared to replicate cells grown on Matrigel) (Fig. 1); 5 ± 3% of the total cells developed in the absence of Matrigel had both eosinophil and basophil granules, 25 ± 2% had eosinophil granules only, and 11 ± 2% had basophil granules only. The remaining cells grown under either culture condition after 14 d were predominantly mononuclear cells lacking visible granules.

Assessment for Metachromasia and Cyanide-resistant Peroxidase. To further characterize the granules of the cells possessing both eosinophil and basophil granules, these cells were stained with toluidine blue and were assessed for cyanide-resistant peroxidase after 14 d of culture. The hybrid nature of the granulation was supported by the findings that with toluidine blue staining, 88 ± 2% of the nonadherent cells were metachromatic (having basophil-like granules) (Fig. 2 B) (n = 4), and 93 ± 1% stained intensely for cyanide-resistant peroxidase (having eosinophil-like granules) (Fig. 2 C) (n = 3). In contrast, when replicate human umbilical cord blood cells were cultured for 14 d with rhIL-3 and rhIL-5 without Matrigel, 16 ± 2% of the nonadherent cells were metachromatic after staining with toluidine blue (n = 3, P <0.05 compared to replicate cells grown on Matrigel), and 37 ± 9% were positive for cyanide-resistant peroxidase (n = 2; average ± range).

Biochemical Assessment of Hybrid Granulocytes. To assess whether granulocytes exhibiting both eosinophilic and basophilic granules had other specific markers for both eosinophils and basophils, the transcription of EDN (eosinophil marker) and histamine content (basophil marker) were measured. Hy-

Figure 2. 14-d cord blood-derived hybrid granulocytes stained (A) with Wright's and Giemsa stains showing individual eosinophil (right arrow) and basophil (left arrow) granules (×100), (B) with toluidine blue (×60), and (C) for cyanide-resistant peroxidase (×60).
bridization signals for EDN were strong at 10 and 14 d of culture and were consistently greater for cells developed in Matrigel-coated flasks than in uncoated flasks (Fig. 3). By 14 d in culture, 96% of the population that was developed on Matrigel and used to prepare the RNA had both eosinophil and basophil granules, while only 8% of the cells developed without Matrigel had both granule types.

By radioimmunoassay, preparations of cells maintained in medium supplemented with rhIL-3 and rhIL-5 in the presence of Matrigel contained 78 ± 16 ng histamine/10^6 cells (n = 6). By staining with Wright's and Giemsa stains, these preparations contained 81 ± 2% hybrid granulocytes and 1 ± 0.3% basophils. No mast cells were present. In contrast, 5 × 10^6 freshly isolated peripheral blood eosinophils of 98% purity did not contain measurable histamine.

**Ultrastructural Analysis of Hybrid Granulocytes.** For transmission electron microscopy, cells developed from cord blood cells by culture for 14 d in medium supplemented by rhIL-3 and rhIL-5 on a bed of Matrigel were examined. More than 80% of the cells were hybrids by Wright's and Giemsa staining. 8 of the 10 cells examined had reticular structures consistent with basophil granules and maturing crystalloid cores consistent with eosinophil granules (11) (Fig. 4).

**Analysis of the Role of Hematopoietic Cytokines in Maintaining the Viability of 14-d Cord Blood-derived Granulocytes**

**Cell Viability during Culture after Cytokine Removal.** To assess whether eosinophil hematopoietins or Matrigel was responsible for maintaining a cell population with mixed granules, the staining characteristics of these cells cultured in the absence of cytokines and extracellular matrix proteins were compared with those of a replicate population maintained under the original culture conditions. Cells developed in vitro from cord blood progenitors cultured for 14 d with rhIL-3 and rhIL-5 on Matrigel were resuspended in RPMI 1640 with 10% FCS only and cultured for an additional 6 d in a plastic vessel without Matrigel. After 6 d in culture (total of 20 d), 93 ± 2% of these cells contained eosinophil granules only, 3 ± 2% contained basophil granules only, none were hybrids, and 4 ± 3% were mononuclear cells lacking visible granules (Fig. 5 C) (n = 2; average ± range). In contrast, of the cells maintained for an additional 6 d in RPMI 1640 supplemented with rhIL-3 and rhIL-5 on a layer of Matrigel, 68 ± 0% were hybrids exhibiting both eosinophil and basophil granules (Fig. 5 B). An additional 27 ± 2% had eosinophil granules only, 2 ± 1% had basophil granules only, and 3 ± 2% lacked visible granules (n = 2, average ± range).

The survival of the 14-d hybrid granulocytes in the absence of exogenous cytokines was remarkably extended in comparison to the survival of freshly isolated eosinophils from
peripheral blood of normal donors (n = 3) (Fig. 6). The survival of the 14-d granulocytes in the absence of exogenous rhIL-5 was 84 ± 6% at 5 d and was not significantly different from replicates cultured with incremental doses of rhIL-5. In contrast, <10% of freshly isolated peripheral blood eosinophils survived for 96 h under the same culture conditions.

**Elaboration of Eosinophilopoietic Cytokine(s) by Hybrid Granulocytes.** After washing the 14-d hybrid granulocytes, their conditioned medium was harvested after 48 h of culture in RPMI 1640 with 10% FCS alone. An average of 78 ± 8% of freshly isolated peripheral blood eosinophils survived for 96 h when cultured in RPMI 1640 supplemented with 25% conditioned medium (n = 3). 13% of these freshly isolated peripheral blood eosinophils were viable in RPMI 1640 with FCS alone, whereas 93 ± 2% were viable in the presence of 10 pM rhIL-5.

To seek the identity of the hybrid granulocyte-derived viability-sustaining activity, neutralization bioassays were used with polyclonal monospecific antibodies directed against the

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**Figure 5.** Staining with Wright's and Giemsa stains of cord blood-derived granulocytes showing their hybrid nature at 14 d of culture (A) and their differentiation with an additional 6 d of culture in the presence (B) or absence (C) of rhIL-3, rhIL-5, and Matrigel.

**Figure 6.** Exclusion of trypan blue by 14-d cord blood–derived hybrid granulocytes maintained for up to 5 d in RPMI 1640 with FCS alone (●) or in RPMI 1640 supplemented with 0.01 (●), 0.1 (▲), 1 (●), 10 (+), and 100 (●) pM rhIL-5. The exclusion of trypan blue by freshly isolated peripheral blood eosinophils maintained in RPMI 1640 with FCS alone is indicated by (+).

**Figure 7.** Exclusion of trypan blue by freshly isolated peripheral blood eosinophils cultured for 96 h in 25% conditioned medium (CM) from 14-d cord blood–derived cultured cells in the absence and presence of neutralizing antibodies against IL-3, IL-5, and GM-CSF. Right-hand column shows viability of eosinophils maintained in RPMI 1640 alone for 96 h.
three eosinophil hematopoietic cytokines (Fig. 7). 25% conditioned medium alone sustained the viability of 78 ± 3% of freshly isolated peripheral blood eosinophils after 96 h in culture. This proportion was not altered by the addition of 100 μg/ml antibody against IL-3 (73 ± 2% viable cells) or IL-5 (71 ± 1% viable cells). In contrast, 50 μg/ml of anti-GM-CSF antibody reduced the viable population to 27 ± 3% (P < 0.01) as compared to 13 ± 7% for eosinophils maintained in enriched medium lacking exogenous cytokines or conditioned medium (n = 3).

Discussion

This study of eosinophil development from cord blood precursors has unexpectedly revealed that a hybrid granulocyte with eosinophil and basophil granules predominated after cell culture for 14 d, and that these hybrid granulocytes are endowed with an autocrine viability-sustaining capacity by virtue of their elaboration of GM-CSF. The acquisition of granules by cord blood granulocyte precursors was accelerated by ~1 wk in cell populations that were developed on Matrigel in the presence of rhIL-3 and rhIL-5 when compared with replicate cells cultured in the presence of cytokines alone (Fig. 1). Furthermore, the phenotypic homogeneity was greatly increased by the presence of Matrigel. Indeed, staining with Wright's and Giemsa stains showed both eosinophilic and basophilic granules in 90% of these cells after 14 d in culture, as recorded in the time-dependent generation of these hybrids.

The predominant development by day 14 of granulocytes with hybrid eosinophil/basophil-staining characteristics was shown with Wright's and Giemsa staining. This was independently confirmed by the observation that 88% of these cells exhibited granule metachromasia when stained with toluidine blue and >90% contained a cyanide-resistant peroxidase, as shown for individual cells in Fig. 2, B and C. The recognition of granule markers specific for basophils (histamine) and for eosinophils (RNA transcripts encoding EDN) supports the presence of two granule phenotypes although it does not confirm their simultaneous occurrence in individual cells (Fig. 3). The hybrid nature of the granule population in the 14-d cultured cells was also confirmed by transmission electron microscopy, demonstrating the coexistence of immature eosinophil granules and basophil granules in the same cells (Fig. 4). Thus, the hybrid eosinophil/basophil granulocytes recognized in patients with acute and chronic myelogenous leukemias (12, 14) are the predominant cellular phenotype in cultures of cord blood mononuclear cells maintained for 14 d in the presence of rhIL-3, rhIL-5, and Matrigel.

Denburg and colleagues (10) reported that clonally derived hematopoietic colonies grown from peripheral blood precursors in semisolid methylcellulose supplemented with Mo-conditioned medium contained mixtures of eosinophils and basophils. 5–48% of the cells in these colonies had both granule types. We observed that when exogenous cytokines and Matrigel were removed from the 14-d cultures and culture was continued in RPMI 1640 with 10% FCS alone for 6 d, 93% of the cells became eosinophils and this population exhibited a greater degree of maturity based on criteria of granule staining properties, cell size, and nuclear morphology than did replicate cells that were maintained in the presence of rhIL-3, rhIL-5, and Matrigel (Fig. 5). Those maintained with rhIL-3, rhIL-5, and Matrigel progressed to only 27% eosinophils, with 68% remaining hybrid granulocytes. There were not significant numbers of basophils in our culture system, either with or without exogenous cytokines. We therefore place these hybrids as intermediates capable of developing into mature eosinophils (Fig. 5).

The mechanism by which Matrigel exerted its effects on granule development is unclear. Both embryonic stem cells and mature eosinophils possess specific receptors for laminin, fibronectin, and other matrix components (30–32). The receptor-mediated interaction of hematopoietic stem cells with matrix components may result in signal transduction and expression of lineage-specific genes as demonstrated for mammary epithelium, hepatocytes, and other cell types (21–24, 33). Alternatively, extracellular matrix proteins can specifically bind and stabilize growth factors that may ensure the exposure of stem cells to optimal local concentrations of cytokines (34). Furthermore, nerve growth factor is a minor constituent of Matrigel. This cytokine has synergistic activity with GM-CSF (35) and with Mo-conditioned medium (36) in stimulating growth and differentiation of granulocyte colonies, particularly of the eosinophil/basophil lineage.

The progression of hybrid granulocytes to eosinophils after exogenous cytokines and Matrigel were removed from the cultures implies an autoregulatory capacity for maintenance of viability. As shown in Fig. 6, ~85% of these cells excluded trypan blue during the course of a 5-d experiment, and little difference in viability existed between cells maintained in the presence or in the absence of rhIL-5. We found that hybrid granulocyte-conditioned medium contained an eosinophil viability-sustaining activity for freshly isolated peripheral blood eosinophils and identified it immunochimically as bioactive GM-CSF (Fig. 7). 25% conditioned medium harvested from 14-d hybrid granulocytes after washing and culture for 48 h without added cytokines sustained the viability of peripheral blood eosinophils for 96 h at 78% as compared to 13% in RPMI 1640 with 10% FCS alone. The viability was reduced to 27% by neutralization with anti-GM-CSF antibody, a reduction of 78% in viability-sustaining activity, whereas anti–IL-3 or anti–IL-5 had no effect.

Mature eosinophils have been shown to produce GM-CSF under certain circumstances. A subset of eosinophils express GM-CSF mRNA in nasal polyps and in airway biopsies after segmental antigen challenge (37, 38). Furthermore, peripheral blood eosinophils can be induced to express GM-CSF protein by stimulation with calcium ionophore or by culture on fibronectin-coated plates (39–41). These observations have prompted speculation that certain eosinophils may have autocrine capabilities in inflammatory disease. Our observation that bioactive GM-CSF is constitutively expressed by hybrid granulocytes suggests that this cellular phenotype might be capable of regulating lineage viability as well as growth and
phenotype may also prolong the viability and stimulate the
differentiation in an autocrine fashion. Furthermore, this
phenotype may also prolong the viability and stimulate the
function of other cells responsive to GM-CSF in a paracrine
fashion.

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References

1. Ackerman, S.J., G.M. Kephart, T.M. Habermann, P.R. Greipp, and G.J. Gleich. 1983. Localization of eosinophil granule major basic protein in human basophils. J. Exp. Med. 158:946–961.
2. Ackerman, S.J., G.J. Weil, and G.J. Gleich. 1982. Formation of Charcot-Leyden crystals by human basophils. J. Exp. Med. 155:1597–1609.
3. MacGlashan, D.W., Jr., S.P. Peters, J. Warner, and L.M. Lichtenstein. 1986. Characteristics of human basophil sulfidopeptide leukotriene release: releasability defined as the ability of the basophil to respond to dimeric cross-links. J. Immunol. 136:2213–2239.
4. Weller, P.F., C.W. Lee, D.W. Foster, E.J. Corey, K.F. Austen, and R.A. Lewis. 1983. Generation and metabolism of 5-lipoxygenase pathway products by human eosinophils: predominant production of leukotriene C4. Proc. Natl. Acad. Sci. USA. 80:7626–7630.
5. Owen, W.F., R.J. Soberman, T. Yoshimoto, A.L. Sheffer, R.A. Lewis, and K.F. Austen. 1987. Synthesis and release of leukotriene C4 by human eosinophils. J. Immunol. 138:532–538.
6. Lopez, A.F., J.M. Eglinton, A.B. Lyons, P.M. Tapley, L.B. To, L.S. Park, S.C. Clark, and M.A. Vadas. 1990. Human interleukin-3 inhibits the binding of granulocyte-macrophage colony-stimulating factor and interleukin-5 to basophils and strongly enhances their functional activity. J. Cell. Physiol. 145:69–77.
7. Lopez, A.F., M.A. Vadas, J.M. Woodcock, S.E. Milton, A. Lewis, M.J. Elliott, D. Gillis, R. Ireland, E.Olwell, and L.S. Park. 1991. Interleukin-5, interleukin-3, and granulocyte-macrophage colony-stimulating factor cross-compete for binding to cell surface receptors on human eosinophils. J. Biol. Chem. 266:24741–24747.
8. Bain, B.J. 1989. Blood Cells. A Practical Guide. J.B. Lippincott Co., Philadelphia. 322 pp.
9. Jublin, L., and G. Michaelson. 1977. A new syndrome characterized by absence of eosinophils and basophils. Lancet. ii:1233–1235.
10. Denburg, J.A., S. Telizyn, H. Messner, B.L.N. Jamal, S.J. Ackerman, G.J. Gleich, and J. Bienenstock. 1985. Heterogeneity of human peripheral blood eosinophil-type colonies: evidence for a common basophil-eosinophil progenitor. Blood. 66:312–318.
11. Dvorak, A.M., H. Saito, P. Estrella, S. Kissell, N. Arai, and T. Ishizaka. 1989. Ultrastructure of eosinophils and basophils stimulated to develop in human cord blood mononuclear cell cultures containing recombinant interleukin-5 or interleukin-3. Lab. Invest. 61:116–132.
12. Weil, S.C., and M.A. Hrisinko. 1987. A hybrid eosinophil-basophil granulocyte in chronic granulocytic leukemia. Am. J. Clin. Pathol. 87:65–70.
13. Poch, T., F. Hermansky, and V. Lodrova. 1973. A contribution to the simultaneous appearance of basophilic and eosinophilic granules in chronic myelogenous leukemia. Neoplasma. 20:413–417.
14. Le Beau, M.M., R.A. Larson, M.A. Bitter, J.W. Vardiman, H.M. Golomb, and J.D. Rowley. 1983. Association of an inversion of chromosome 16 with abnormal marrow eosinophils in acute myelomonocytic leukemia. N. Engl. J. Med. 309:630–636.
15. Bitter, M.A., M.M. Le Beau, R.A. Larson, M.C. Rosner, H.M. Golomb, J.D. Rowley, and J.W. Vardiman. 1984. A morphologic and cytochemical study of acute myelomonocytic leukemia with abnormal marrow eosinophils associated with Inv(16) (p13q22). Am. J. Clin. Pathol. 81:733–741.
16. Lapidot, T., C. Sirard, J. Vormoor, B. Murdoch, T. Hoang, J. Caceres-Cortes, M. Minden, B. Paterson, M.A. Galigiuri, and J.E. Dick. 1994. A cell initiating human acute myeloid leukemia after transplantation into SCID mice. Nature (Lond.). 367:645–648.
17. Saito, H., K. Hatake, A.M. Dvorak, K.M. Leiferman, A.D. Donnenberg, N. Arai, K. Ishizaka, and T. Ishizaka. 1988. Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. Proc. Natl. Acad. Sci. USA. 85:2288–2292.
18. Leary, A.G., and M. Ogawa. 1984. Identification of pure and mixed basophil colonies in culture of human peripheral blood and bone marrow cells. Blood. 64:78–83.
19. Clutterbuck, E.J., E.M. Hirst, and C.J. Sanderson. 1989. Human interleukin 5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. Blood. 73:1504–1512.
20. Walsh, G.M., A. Hartnell, R. Mogbel, O. Cormwell, L. Nagy, B. Bradley, T. Furitsu, T. Ishizaka, and A.B. Kay. 1990. Receptor expression and functional status of human eosinophils derived from umbilical cord blood mononuclear cells. Blood. 76:105–111.
21. Barcellos-Hoff, M.H., J. Aggelet, T.G. Ram, and M.J. Bissell. 1989. Functional differentiation and aleolar morphogenesis of primary mammary cultures on reconstituted basement mem-
branes. Development (Camh). 105:223–235.
22. Li, M.L., J. Aggerler, D.A. Farson, C. Hatier, J. Hassell, and M.J. Bissell. 1987. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. Proc. Natl. Acad. Sci. USA. 84:136–140.
23. Scheutz, E.G., J.D. Scheutz, B. May, and P.S. Guzelian. 1990. Regulation of cytochrome P450b.e and A-450p gene expression by growth hormone in rat hepatocytes cultured on a reconstituted basement membrane. J. Biol. Chem. 265:1188–1192.
24. Nicotia, R.F., and A. Ottinetti. 1990. Modulation of microvascular growth and morphogenesis by reconstituted basement membrane gel in three-dimensional cultures of rat aorta: a comparative study of angiogenesis in in Matrigel<collagen<fibrin and plasma clot. In Vitro Cell & Dev. Biol. 26:119–128.
25. Wantanabe, I., S. Donahue, and N. Hoggatt. 1967. Method for electron microscopic studies of circulating human leukocytes and observations of their fine structure. J. Ultrastruct. Res. 20:366–382.
26. Rosenberg, H.F., D.G. Tenen, and S.J. Ackerman. 1989. Molecular cloning of the human eosinophil-derived neurotoxin: a member of the ribonuclease gene family. Proc. Natl. Acad. Sci. USA. 86:4460–4464.
27. Hansel, T.T., I.J. De Vries, T. Iff, S. Rihs, M. Wandzilak, S. Betz, K. Blaser, and C. Walker. 1991. An improved immunomagnetic procedure for the isolation of highly purified human blood eosinophils. J. Immunol. Methods. 145:105–110.
28. Owen, W.F., M.E. Rothenberg, J. Petersen, P.F. Weller, D.S. Silberstein, A.L. Sheffer, R.L. Stevens, R.J. Soberman, and K.F. Austen. 1989. Interleukin 5 and phenotypically altered eosinophils in the blood of patients with the idiopathic hypereosinophilic syndrome. J. Exp. Med. 170:343–348.
29. Her, E., J. Frazer, K.F. Austen, and W.F. Owen. 1992. Eosinophil hematopoietins antagonize the programmed cell death of eosinophils: cytokine and glucocorticoid effects on eosinophils maintained by endothelial cell conditioned medium. J. Clin. Invest. 88:1982–1987.
30. Georas, S.N., B.W. McIntyre, M. Ebisawa, J.L. Bednarczyk, S.A. Sterbinski, R.P. Schleimer, and B.S. Bochner. 1993. Expression of a functional laminin receptor (alpha 6 beta 1, very late antigen 6) on human eosinophils. Blood. 82:2872–2879.
31. Williams, D.A., M. Rios, C. Stephens, and V. Patel. 1991. Fibronectin and VLA-4 in hematopoietic stem cell-microenvironmental interactions. Nature (Lond). 352:438–441.
32. Cooper, H.M., R.N. Tamura, and V. Quaranta. 1991. The major laminin receptor of mouse embryonic stem cells is a novel isoform of the alpha 6 beta 1 integrin. J. Cell Biol. 115:843–850.
33. Nowak, R. 1994. Cell biologists get the message. Science (Wash. DC). 263:30–31.
34. Flauemehaft, R., and D.B. Rifkin. 1992. The extracellular regulation of growth factor action. Mol. Biol. Cell. 3:1057–1065.
35. Tsuda, T., D. Wong, J. Dolovich, J. Bienenstock, J. Marshall, and J.A. Denburg. 1991. Synergistic effects of nerve growth factor and granulocyte-macrophage colony-stimulating factor on human basophilic cell differentiation. Blood. 77:971–979.
36. Matsuda, H., M.D. Coughlin, J. Bienenstock, and J.A. Denburg. 1988. Nerve growth factor promotes human hematopoietic colony growth and differentiation. Proc. Natl. Acad. Sci. USA. 85:6508–6512.
37. Ohno, I., R. Lea, S. Finotto, J. Marshall, J. Denburg, J. Dolovich, and M. Jordan. 1991. Granulocyte/macrophage colony-stimulating factor (GM-CSF) gene expression by eosinophils in nasal polyposis. Am. J. Respir. Cell Mol. Biol. 5:505–510.
38. Broide, D.H., M. Paine, and G.S. Firestein. 1992. Eosinophils express interleukin 5 and granulocyte-macrophage colony-stimulating factor mRNA at sites of allergic inflammation. J. Clin. Invest. 90:1414–1424.
39. Kita, H.T., Y. Okubo, D. Weiler, J.S. Abrams, and G. Gleich. 1991. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. J. Exp. Med. 174:745–748.
40. Moqbel, R., Q. Hamid, S. Ying, J. Barkans, A. Hartnell, A. Tsicopoulos, A.J. Wardlaw, and A.B. Kay. 1991. Expression of mRNA for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. J. Exp. Med. 174:749–752.
41. Anwar, A.R.F., R. Moqbel, G.M. Walsh, A.B. Kay, and A.J. Wardlaw. 1993. Adhesion to fibronectin prolongs eosinophil survival. J. Exp. Med. 177:839–843.