CONCISE REPORT

Acquisition of Formyl Peptide Receptors During Normal Human Myeloid Differentiation

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By using a newly developed immune rosetting technique to isolate highly purified populations of myeloid precursor cells from normal human bone marrow and then inducing their differentiation with granulocyte and macrophage colony-stimulating factors (G/M-CSFs) in vitro, we studied the surface expression of chemotactic peptide receptors as the cells matured from the stage of the myeloblast to that of the mature, segmented neutrophil. We used ethylene glycol bis(succinimidyl succinate) to link N-formyl-Nle-Leu-Phe-Nle-[3H]odo-Tyr-Lys to chemotactic peptide receptors on the surface of myeloid cells at sequential stages of maturation and then determined the density of receptor-radioligand complexes by autoradiography after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Specific, saturable formyl peptide receptors were not detectable at the level of the myeloblast but gradually emerged through progressive stages of neutrophil maturation. The specific receptors for formyl peptide that appeared during cellular maturation had a mol wt of 55 to 70 kiloDalton (kD), corresponding to those present on the surface of peripheral blood neutrophils, and binding of the radioligand was highly specific in that it was completely inhibited by a 1,000-fold excess of F-Met Leu Phe. These data correlate with and provide insight into our recent observation that F-Met Leu Phe-induced membrane depolarization and transient increases in cytosolic free calcium are gradually acquired as neutrophils mature. This report represents to our knowledge the first description of the maturational development of chemotactic peptide receptor expression in normal human myeloid cells.

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MATERIALS AND METHODS

Isolation of myeloid precursor cells. Myeloid precursor cells from the stage of the committed G/M progenitor cell (CFUc) through the stage of the myeloblast were enriched ~100-fold using an immune rosetting technique that we previously described in detail. Monoclonal antibodies used to deplete differentiated lymphoid and myeloid cells from marrow cell suspensions included a-MY8 and a-Mol (panmyeloid), a-MY4 (monocyte), a-B1 (B lymphocyte), a-T3 and a-T11 (T lymphocyte), 31C6 (NK cell and basophil, kindly provided by Drs J. Ritz and T. Hercend, Dana-Farber Cancer Institute, Boston) and a-glycophorin A (erythroid). Cells binding these antibodies were then eliminated on sodium metrizoate-Ficoll gradients after being allowed to form rosettes with sheep erythrocytes coated with rabbit anti-murine immunoglobulin.

Differentiation of myeloid precursor cells in liquid suspension cultures. One to 2 x 10^6 isolated myeloid cells were suspended in McCoy's 5A medium (Gibco, Grand Island, NY) containing 10% heat-inactivated fetal calf serum, penicillin (50 IU/mL), and streptomycin (50 µg/mL), at a concentration of 5 x 10^6 cells per milliliter. As a source of G/M-CSFs, 5% GCT medium (Gibco) and 5% medium conditioned by the Mo cell line (Gift of Dr Steven Clark, Genetics Institute, Cambridge, MA) were added, and the culture was incubated at 37°C in a humid incubator containing 5% CO_2 for ten days. At appropriate intervals, total and differential cell counts were performed.

Affinity labeling of chemotactic peptide receptors. Affinity labeling of formyl peptide receptors on whole cells was performed as previously described. All labeling experiments were carried out.
either on whole fresh cells or on whole cells fixed with paraformaldehyde. For experiments represented by Fig 1, aliquots of cells were removed from the culture at intervals between zero and ten days, fixed in 1% paraformaldehyde, washed in phosphate-buffered normal saline, pH 7.4 (PBS), and then stored at 4°C until affinity labeling. For the experiments represented in Fig 2, cells were not fixed in paraformaldehyde and stored but instead were taken fresh from the cultures and affinity labeled immediately after washing. In brief, the hexapeptide analogue of F-Met Leu Phe, N-formyl-Leu-Phe-Nle-Tyr-Lys (Bachem, Inc, Torrence, CA) was labeled with 125Iiodine (N-formyl-Leu-Phe-Nle-[125I]iodo-Tyr-Lys). Affinity labeling of fixed or fresh whole cells was performed using 50 nmol/L N-formyl-Leu-Phe-Nle-[125I]iodo-Tyr-Lys and ethylene glycol bis(succinimidyl succinate) (Pierce Chemical Co, Rockford, IL). Labeled cells were extracted with Triton X-100 in tris-glycine buffer and subjected to SDS-PAGE and autoradiography as previously described. In a control study, we determined that paraformaldehyde fixation of granulocytes did not decrease affinity labeling of receptors, did not alter the molecular weight of receptor detected, and did not inhibit the ability of the detergent to solubilize the receptor. However, there was some decrease of receptor that could be affinity labeled after granulocytes were fixed and stored at 4°C for eight days, but no change in the apparent molecular weight of receptor labeled.

RESULTS

The total and differential cell counts obtained using the technique for myeloid cell isolation and in vitro differentiation that we employed in this report have been described previously in detail. Immediately after the cells were isolated from the bone marrow, the vast majority (~85%) were myeloblasts or morphologically unidentifiable, immature hemic precursors, as assessed by Wright-Giemsa staining. After 24 hours in culture, a substantial number of promyelocytes became evident (~55%), with very few cells beyond that stage of development. Thereafter, a progressive wave of maturation took place, so that by day 8, most of the cells (~75%) were postmitotic neutrophils, and by day 10, ~65% were fully segmented neutrophils.

The acquisition of the formyl peptide receptor complex at sequential stages of cellular maturation in a representative experiment from a single donor is shown in Fig 1. Each lane shows the binding on successive days of iodinated hexapeptide to 10⁶ cells that were removed from the culture, fixed with paraformaldehyde, and studied by covalent affinity labeling. On day 0, at which time very few cells more mature than the myeloblast were present, binding was undetectable under these conditions. On day 1, when the predominant type of cell in the culture was the promyelocyte, a single band appeared that had an apparent mol wt of ~55,000. Labeling of this band could be completely inhibited by a 1,000-fold excess of F-Met Leu Phe (data not shown). By day 3, when considerable maturation past the promyelocyte stage had taken place, an additional band with a higher mol wt was visible that also represented specific saturable receptor. Thereafter, as the cells matured through the stage of the mature granulocyte, both bands became progressively more intense.

Since storage of fixed cells leads to some loss of detectable formyl peptide receptor, freshly isolated live day 0 myeloid precursors were affinity labeled and analyzed without fixation or storage. These were compared with similarly treated day 8 cultures derived from a different donor. Ten times more labeled cells (10⁶) were loaded in each lane of the experiment shown in Fig 2 than were analyzed in each lane in Fig 1 in order to increase further the sensitivity of detection of any specific receptor for formyl peptide present in myeloblasts. Under these conditions, a broad band with average mol wt of ~80 kD (Fig 2, lane A), which was only slightly decreased by a 1,000-fold excess of F-Met Leu Phe (Fig 2,
of human granulocytes antecede the metabolic response to surface chemotactic factor-induced release of membrane calcium in rabbit neutrophils. i Biol Chem 254:10700, 1979

In 1980, Niedel et al.14 showed that high affinity formyl peptide receptors were present on a subpopulation of undifferentiated HL-60 cells and that they increased 70- to 100-fold when the cells were caused to differentiate along the granulocyte pathway. The receptor-bearing cells, while varying in number from experiment to experiment, amounted to ~10% of the total number of cells in unstimulated cultures and were said to resemble well differentiated promyelocytes. The number of receptors on the subpopulation of undifferentiated cells ranged from >300 to 15,000 per cell, while after the cells were induced to differentiate, the density increased to ~200,000 per cell.

Our results confirm that the maturational development of formyl peptide receptor expression is similar in normal granulocytes to that predicted by the HL-60 model and provide further insight into the level at which the receptors may first appear. Unlike unstimulated HL-60 cells, the cell fraction that we harvest contains practically no cells with granules, since very few are more mature than myeloblasts.

In 1984, Brown et al.15 showed the development of membrane-potential responsiveness by myeloblast leukemia cells during differentiation of HL-60 cells. Biochem Biophys Res Commun 122:973, 1984

REFERENCES


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