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Chemotactic Activity of Bone Marrow-Derived Macrophages Changes with Time in Culture

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The number of bone marrow-derived macrophages able to respond to chemotactic stimuli varies with time in culture. Chemotactic response was optimal at 2 weeks and may depend on cell maturation or differentiation or both.

Morphological, biochemical, and functional activities of mononuclear phagocytes change with maturation or differentiation or both (2, 4, 5). It is generally believed that the inflammatory macrophages that accumulate at sites of inflammation originate in the bone marrow and are transported via the blood as monocytes (13, 14); monocytes arrive at or are recruited to sites of inflammation and mature or differentiate into effector macrophages. The most probable mechanism for this monocyte recruitment is chemotaxis along a concentration gradient. On their way from bone marrow through the blood to tissue, these immature mononuclear phagocytes could change characteristics and develop into mature macrophages. On the other hand, Bursuker and Goldman proposed that resident and inflammatory macrophages may in fact derive from different precursor stem cells (3). Culture of bone marrow-derived cells has been used to follow the development of macrophage function (3, 12). In this note, we report on changes in chemotactic and phagocytic properties of bone marrow-derived cells at different times in culture.

Bone marrow cells were prepared from femura of adult C3H/HeN male mice (10), 20 to 25 g, obtained from stock at the National Institutes of Health, and cultured as previously described (12). One modification was culture in 250-ml polypropylene centrifuge tubes (no. 25350, Corning Glassworks, Corning, N.Y.). Cells were seeded at a density of 2×10^7 per 10 ml per tube. Tubes were incubated on their sides in moist air with 5% carbon dioxide. Cells were refed every other day from day 5 on with 5 ml of culture medium. Cultured cells were harvested by centrifugation of tubes at $250 \times g$ for 10 min. Adherent cells were detached with a rubber policeman before centrifugation or by rinsing with a pipette. More than 95% of cells recovered were viable as assessed by trypan blue exclusion. Results obtained with these cells were very similar to results obtained with bone marrow macrophages recovered from Teflon-coated dishes (10). Chemotaxis was performed in a 48-well chemotaxis chamber (6). Bottom wells were filled with 25 μl of endotoxin-activated mouse serum (11) diluted in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.). A Nucleopore filter sheet (Neuroprobe Inc., Bethesda, Md; polyvinylpyrrolidone-coated, 10 μm thick, 5-μm hole size) was placed over the wells. Gasket and top plate were assembled, and 20,000 macrophages in 50 μl of culture medium were added to each top well. Chambers were incubated in moist air containing 5% carbon dioxide for 4 h. After incubation, chambers were disassembled and filters were removed. Cells remaining on the top side were wiped off and the filter was air dried and stained in Diff-Quick (Harleco, Gibbstown, N.J.). The number of cells per square millimeter was counted with an image analyzer, and the percentage of input macrophages migrated was calculated in triplicate samples (total filter area available for chemotaxis was 8 mm^2). When neutrophils were present on the bottom of the filter, differential counts were made for each triplicate sample, and the number of migrated macrophages was calculated.

Phatocytosis was quantified by uptake of anti-Forssman immunoglobulin G-coated sheep erythrocytes, labeled with ^{51}Cr as described earlier (11). The numbers represent the total uptake of 2×10^5 cells in each group. In bone marrow cultures grown with L-cell conditioned medium, the number of morphologically defined macrophages increased linearly until days 8 to 10 and then remained constant. From day 9 on, cells were more than 90% macrophages. The percentage of peroxidase-positive macrophages increased until day 4 (Fig. 1), remained constant for 5 days, and then decreased; from day 14 on, no peroxidase-positive cells were found. From days 4 through 10, the only other cells found were polymorphonuclear granulocytes. From

about day 10 on, 100% of the cells were macrophages.

We tested phagocytic and chemotactic properties after various times in culture. Phagocytic activity of bone marrow cells increased with time in culture (Fig. 2). The rate of increase was rapid during the first days of culture and then slowed so that by day 26, these cells were about six times as active as peritoneal exudate macrophages. This is consistent with previously reported observations that the phagocytic capacity of macrophages in culture gradually increases with time (1a).

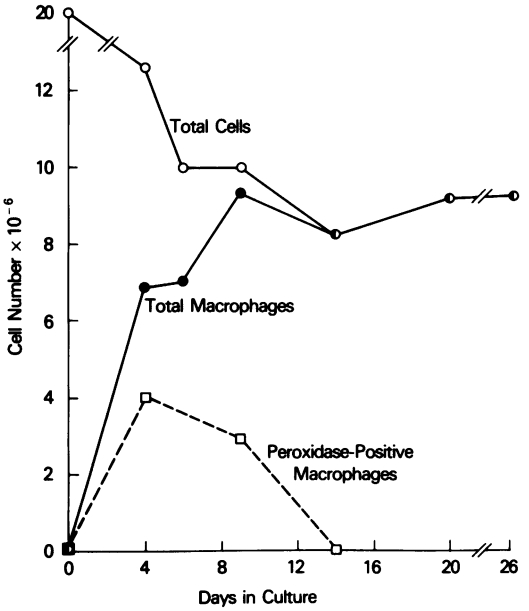

FIG. 1. *Growth of mouse bone marrow cells in vitro.*

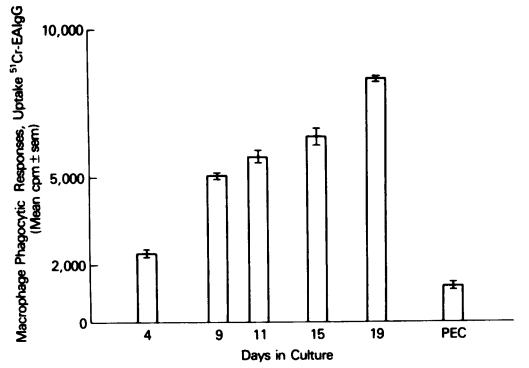

FIG. 2. *Phagocytic responses of bone marrow-derived macrophages. cpm, Counts per minute; sem, standard error of the mean. PEC, Peritoneal exudate cells; ^{51}Cr-EAIgG, sheep erythrocytes labeled with ^{51}Cr and immunoglobulin G antibody.*

The chemotactic activity of bone marrow macrophages also increased dramatically during the first days of culture. Peak activity occurred around day 12 and then decreased to that of resident peritoneal macrophages by day 22 (Fig. 3). No contaminating cells were present at this time. In numerous experiments, we found this peak activity between days 11 and 14, with 70 to 100% of the input macrophages migrating. That chemotactic responses reached a plateau with increasing concentration of attractant suggests that only a subpopulation of macrophages was able to respond (Fig. 4). Changes in chemotaxis by bone marrow macrophages with time in culture, then, must represent changes in this responsive subpopulation. The usual migration of macrophages or granulocytes from a variety of sources has not been more than 20 to 40% of input number (2, 7, 9). The finding that 100% of the bone marrow macrophages can migrate is of great interest. It shows that limited migration is not caused by physical restrictions within the

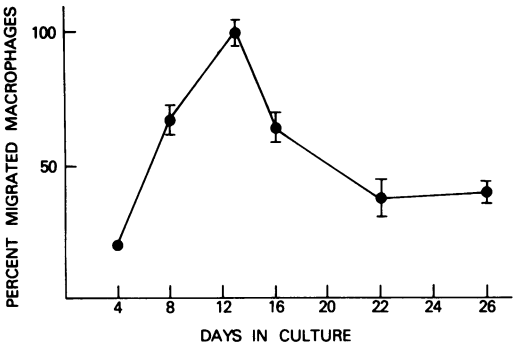

FIG. 3. *Chemotactic responses of bone marrow-derived macrophages. Results are expressed as the percent total input macrophages that migrated to 1:200 endotoxin-activated mouse serum.*

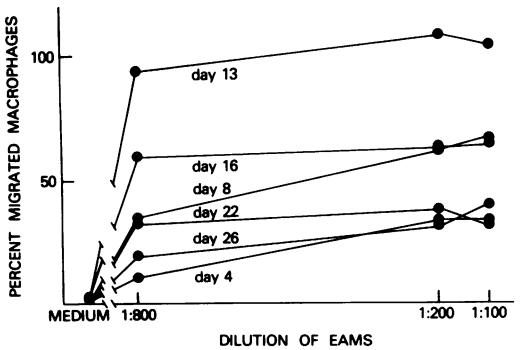

FIG. 4. *Chemotactic dose responses of mouse bone marrow-derived macrophages after various days in culture. Standard errors of the means did not exceed 15%. EAMS, Endotoxin-activated mouse serum.*

assay system but that specific requirements must be met by the macrophages before they are able to express chemotaxis. These requirements could be met by changes in stage of maturation or differentiation or by production of required accessory factors by the cells themselves. The percent migration of mouse resident peritoneal macrophages, for example, can be manipulated by the number of lymphocytes in the cell mixture or simply by cell density (E. J. Leonard and A. H. Skeel, J. Reticuloendothel. Soc., in press). It is of interest that some but not all mouse macrophage cell line cells exhibit a 100% migration (1). The high chemotactic activity of bone marrow cells at certain developmental stages shown in this report provides a possible mechanism by which macrophages needed at inflammation sites are recruited from the bone marrow. This recruitment may be mediated through complement. The complement split product C3e facilitates release of cells from the bone marrow (8), and the complement fragment C5a serves as a strong chemoattractant. Both are generated during inflammation.

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