Distribution of Cells Bearing Receptors for a Colony-stimulating Factor (CSF-1) in Murine Tissues

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ABSTRACT  CSF-1 is a subclass of the colony-stimulating factors that specifically stimulates the growth of mononuclear phagocytes. We used the binding of 125I-CSF-1 at 0°C by single cell suspensions from various murine tissues, in conjunction with radioautography, to determine the frequency of binding cells, their identity, and the number of binding sites per binding cell. For all tissues examined, saturation of binding sites was achieved within 2 h at 2-3 x 10^-10 M 125I-CSF-1. The binding was irreversible and almost completely blocked by a 2 h preincubation with 5 x 10^-10 M CSF-1. 125I-CSF-1 binding was exhibited by 4.3% of bone marrow cells, 7.5% of blood mononuclear cells, 2.4% of spleen cells, 20.5% of peritoneal cells, 11.8% of pulmonary alveolar cells and 0.4% of lymph node cells. Four morphologically distinguishable cell types bound 125I-CSF-1: blast cells; mononuclear cells with a ratio of nuclear to cytoplasmic area (N/C) >1; cells with indented nuclei; and mononuclear cells with N/C ≤1. No CSF-1 binding cells were detected among blood granulocytes or thymus cells. Bone marrow promyelocytes, myelocytes, neutrophilic granulocytes, eosinophilic granulocytes, nucleated erythroid cells, enucleated erythrocytes, and megakaryocytes also failed to bind. The frequency distribution of grain counts per cell for blood mononuclear cells was homogenous. In contrast, those for bone marrow, spleen, alveolar, and peritoneal cells were heterogeneous. The monocytes in blood or bone marrow (small cells, with either indented nuclei or with N/C >1) were relatively uniformly labeled, possessing ~3,000 binding sites per cell. Larger binding cells (e.g., alveolar cells) may possess higher numbers of receptors. It is concluded that CSF-1 binding is restricted to mononuclear phagocytic cells and their precursors and that it can be used to identify both mature and immature cells of this series.

Colony-stimulating factors (CSFs) are growth factors which stimulate the formation of colonies of granulocytes and/or macrophages (3, 14) by precursor cells in hemopoietic tissues (reviewed in reference 13). Four subclasses may be discerned by their preferential effects on neutrophil, eosinophil, neutrophil-macrophage or macrophage colony formation (reviewed in reference 17). CSF-1 is a subclass discriminated from the other subclasses by its detection in subclass-specific radiomuno- and radioreceptor assays (5, 16, 17). L-cell CSF-1 is identical to L-cell macrophage growth factor (19) and specifically stimulates the growth of mononuclear phagocytes and their precursors (17, 18, 20). It is a heavily glycosylated, sialic acid-containing glycoprotein (molecular weight 40,000-86,000) composed of two disulfide-bonded subunits (21, 22). The specific binding of radiolabeled CSF-1 to mononuclear phagocytes and to cell lines derived from mononuclear phagocytic cells has recently been demonstrated (8). Several independent findings indicate that the high affinity CSF-1 binding site described in these studies is the receptor through which the biological effects of CSF-1 are mediated (8, 21).

In this study we examine the distribution, frequency, and morphology of CSF-1 receptor-bearing cells in adult mouse tissues. Binding conditions were chosen to ensure saturation of the cell-surface receptors by 125I-CSF-1 and radioautography was used to identify the binding cells and to determine the number of CSF-1 receptors per cell. We report the existence of a CSF-1 receptor, with common properties, on cells in a variety of tissues and indicate its usefulness as a marker of both mature and immature cells of the mononuclear phagocytic series.

MATERIALS AND METHODS

Preparation of 125I-CSF-1

L-cell CSF-1 was purified as previously described (21, 22). The purity was checked by PAGE, with and without SDS under reducing and nonreducing conditions.
Preparation of Cells

8- to 12-wk-old female C3H/An mice (Cumberland Farms, Nashville, Tenn.) were used exclusively. Unless otherwise stated, the animals were killed by cervical dislocation and the cells suspended in HS-a-HEPES containing 0.1% HEPES (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) in lieu of bicarbonate. The pH was 7.2, with 10% (vol/vol) horse serum (Flow Laboratories, Inc., Rockville, Md.), at -20°C (half-life of biologically active CSF-1 = 50 d). They were used no longer than 3 wk postinduction. Determinations of the specific radioactivites, as described elsewhere (21), were made at the time of each experiment. The mean value was ~106,000 cpm/ng of biologically active CSF-1 protein or 2,900 cpm/fg, corresponding to an average of 1 atom of 125I per 100,000 molecular weight. Molarity conversions are based on an M, for the polypeptide moiety of CSF-1 of 28,000 (S. K. Das and E. R. Stanley, manuscript in preparation (21) and the specific activity of purified CSF-1 (~1.6 x 10^6 U/mg protein) (22).

Radioautographs

Kodak NTB2 (Eastman Kodak Co., Rochester, N. Y.) was heated to 42°C and a thin layer was applied to each slide by dipping. They were enclosed in light-tight slide boxes, containing Drierite (W. A. Hammond Drierite Co., Xenia, Ohio) as desiccant, and exposed at 4°C for 4, 7, or 14 d. Slides were developed at 22°C for 2 min in Kodak D-19 developer, dipped in 0.2 M acetic acid for 30 s and fixed for 3 min in Kodak Rapid Fixer. They were washed in running tap water for 15 min and rinsed in distilled water. The cells were stained in 0.3% (wt/vol) May-Grunwald in methanol, followed by 0.02% (wt/vol) Giemsa in 3 mM sodium phosphate buffer pH 6.8 for 30 min. The emulsion was developed in 20 mM acetic acid in ethanol, rinsed in buffer, and air-dried. Cells were examined with a Zeiss Standard WL Microscope (Carl Zeiss, Inc., N. Y.). The grain threshold was determined with the control cells that had been preincubated with unlabeled CSF-1. At least 1,000 sequential cells were examined in each preparation.

RESULTS

The binding conditions were determined using the macropage cell line J-774.2 as the target cell population. Their ability to bind 125I-CSF-1 at 0°C has been previously demonstrated (8). Saturation of cellular binding was achieved, at concentrations of 125I-CSF-1 >2 x 10^-10 M, within 2 h of incubation at 0°C (Fig. 1a). There was a linear relationship between cpm bound and cell concentration in the range 1 x 10^6 to 1 x 10^7 cells/ml (Fig. 1b). Binding was essentially irreversible as 125I-CSF-1 was removed by washing with cold methanol at 0°C, washed three times in H_2O, air-dried, and then processed for radioautography.

Cellular Binding of 125I-CSF-1

All operations were carried out at 0°C. Cells (usually 5 x 10^6 nucleated cells) in HS-a-HEPES were added to precooled plastic 35-mm tissue culture dishes (Lux Scientific Corp., Newburg Park, Calif.) and allowed to stand for 30 min. In the case of blood mononuclear, alveolar, and peritoneal cells, <5 x 10^6 nucleated cells per dish were used because of the limited cell yield per mouse. Stage I I-cell CSF-1 (22) was added to control dishes (final concentration 5 x 10^-10 M) to saturate all CSF-1 binding sites before incubation with 125I-CSF-1 (21). The remaining dishes received an equal volume of HS-a-HEPES. The contents of each dish were thoroughly mixed and allowed to incubate for 2 h.

125I-CSF-1 was then added to all dishes with mixing (final volume 1 ml) and the dishes incubated for a further 2 h. For all cell populations studied, saturation was observed at the 125I-CSF-1 concentration routinely used for binding (5 x 10^-10 M). The cells were then thoroughly resuspended and unbound 125I-CSF-1 was removed by layering 0.8 ml of the suspension over 3 ml of filtered horse serum in a 12 x 75-mm polypropylene tube (Falcon #2053) and centrifuging (500 g, 15 min). The supernate was removed by aspiration. The cell pellet was resuspended in 0.2 ml medium and quantitatively transferred to a new tube. 0.3 ml of medium was added and the tube was centrifuging (500 g, 10 min) immediately or after standing for 24 h. The supernate was discarded and the cell pellet on ice was counted for 125I in an LKB 1280 Ultrogamma counter (LKB Instruments, Inc., Rockville, Md.). Cells were resuspended in fetal calf serum and ~1 x 10^6 cells were smeared on precleaned glass slides. The cells were fixed in cold methanol for 15 min, washed three times in H_2O, air-dried, and then processed for radioautography.

Bone marrow and spleen cells were used exclusively. Unless otherwised stated, the animals were killed by cervical dislocation and the cells suspended in HS-a-HEPES containing 0.1% HEPES (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) in lieu of bicarbonate. The pH was 7.2, with 10% (vol/vol) horse serum (Flow Laboratories, Inc., Rockville, Md.). At -20°C (half-life of biologically active CSF-1 = 50 d). They were used no longer than 3 wk postinduction. Determinations of the specific radioactivites, as described elsewhere (21), were made at the time of each experiment. The mean value was ~106,000 cpm/ng of biologically active CSF-1 protein or 2,900 cpm/fg, corresponding to an average of 1 atom of 125I per 100,000 molecular weight. Molarity conversions are based on an M, for the polypeptide moiety of CSF-1 of 28,000 (S. K. Das and E. R. Stanley, manuscript in preparation (21) and the specific activity of purified CSF-1 (~1.6 x 10^6 U/mg protein) (22).

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Fig. 2 shows saturation binding curves for bone marrow and spleen cells. Binding sites were saturated at concentrations between 2 and \(3 \times 10^{-10} \text{M}\). Saturation was achieved within 2 h at 0°C. The binding was essentially irreversible for all the tissues studied as further incubation of the loaded cells for 24 h at 0°C in CSF-1-free medium resulted in no loss of labeled cells of particular morphology were observed.

Labeled and unlabeled cells of a particular morphology were characterized as monocytes (see Discussion). Analysis of all three tissues represented in Fig. 4 failed to reveal any other striking correlation between cellular morphology and grain counts per cell. However, the binding cells in the pulmonary alveolar tissue were heterogeneous in most populations examined, including bone marrow (Fig. 4a), alveolar (Fig. 4c) and spleen and peritoneal cells (not shown). However, as seen in Fig. 4b, blood mononuclear cells were relatively homogeneous in terms of the number of grains per cell (10–45 grains/cell, 3,000 binding sites/cell). They consisted of mononuclear cells with N/C \(>1\) (68%) and cells with accentuated nuclear indentations (32%) (Table II), both of which were characterized as monocytes (see Discussion). Analysis of all three tissues represented in Fig. 4 failed to reveal any other striking correlation between cellular morphology and grain counts per cell. However, the binding cells in the pulmonary alveolar tissue were heterogeneous in most populations examined, including bone marrow (Fig. 4a), alveolar (Fig. 4c) and spleen and peritoneal cells (not shown). However, as seen in Fig. 4b, blood mononuclear cells were relatively homogeneous in terms of the number of grains per cell (10–45 grains/cell, 3,000 binding sites/cell). They consisted of mononuclear cells with N/C \(>1\) (68%) and cells with accentuated nuclear indentations (32%) (Table II), both of which were characterized as monocytes (see Discussion). Analysis of all three tissues represented in Fig. 4 failed to reveal any other striking correlation between cellular morphology and grain counts per cell. However, the binding cells in the pulmonary alveolar

Fig. 1 Binding of \(^{125}\text{I}\)-CSF-1 to J-774.2 cells. (a) Kinetics of \(^{125}\text{I}\) binding by \(0.4 \times 10^6 \text{cells at 0°C in the presence of } 3 \times 10^{-10} \text{M }^{125}\text{I}\)-CSF-1. Points represent means of duplicate values. (b) \(^{125}\text{I}\) binding as a function of cell number. Cells were incubated for 2 h at 0°C with \(4.2 \times 10^{-10} \text{M }^{125}\text{I}\)-CSF-1 alone (○) or after a 2 h preincubation with \(5 \times 10^{-10} \text{M unlabeled CSF-1 (●)} \) at 0°C. Net binding is the difference between the two lines. Points represent means of duplicate values.
preparation were larger and bound more $^{125}$I-CSF-1 per cell than the blood mononuclear binding cells.

**DISCUSSION**

A prior report demonstrated stable binding of $^{125}$I-CSF-1 to mouse peritoneal exudate cells at 0°C (8). At 37°C, $^{125}$I-CSF-1 is internalized and destroyed (21). Binding at 0°C was therefore used to determine the frequency of binding cells in various tissues and to quantitate the number of unoccupied cell surface CSF-1 binding sites per cell in these tissues. Despite variations in the number of sites per cell between and often within the tissue examined, the CSF-1 receptor exhibited similar characteristics in all cell populations; the saturation binding conditions were found to be similar and binding was essentially irreversible.

In all tissues examined, CSF-1 binding was restricted to four cell types: blast cells; mononuclear cells with N/C > 1; cells with indented nuclei; and mononuclear cells with N/C ≤ 1. CSF-1 binding blast cells were found in bone marrow, and in a higher proportion among peritoneal and alveolar cells (in the latter populations many "blast" cells were possibly multinucleated giant cells). Mononuclear cells (N/C > 1) binding CSF-1 (cells with lymphocytelike morphology) were found in all tissues. Binding cells with indented nuclei included promonocytes (7) and monocytes. Mononuclear cells (N/C ≤ 1) (cells with a macrophagelike morphology) that bound CSF-1 were found among peritoneal, alveolar, and lymph node cells. In all tissues studied, granulocytes (promyelocytes to mature neutrophils and eosinophils), nucleated and enucleated erythroid cells, and megakaryocytes failed to bind CSF-1. Furthermore, the low frequency of binding cells among lymphocytelike cells (N/C > 1) in the blood mononuclear, thymic, and lymph node cell populations makes it very unlikely that B or T lymphocytes possess CSF-1 receptors. Thus the morphology of the binding cells is consistent with the conclusion that they are either mononuclear phagocytes or undifferentiated mononuclear phagocytic precursor cells. Other kinds of evidence support this conclusion. (a) Although many of the binding cells cannot be identified (especially the blasts and mononuclear cells with N/C > 1), all of the identifiable mononuclear phagocytic cells in most populations bind $^{125}$I-CSF-1. All blood mononuclear cells with an accentuated nuclear indentation (classical blood monocytes) bound $^{125}$I-CSF-1. It was reported (8) that >95% of adherent peritoneal cells (peritoneal macrophages) bound $^{125}$I-CSF-1 and that >98% of a population of adherent peritoneal exudate cells (>99% of which displayed Fc-mediated sheep erythrocyte phagocytosis) bound $^{125}$I-CSF-1. (b) In this study, CSF-1 binding cells were preferentially distributed among tissues known to contain mononuclear phagocytic cells (25). (c) The frequencies of binding cells (Table I) in the tissues examined are in general agreement with the previously reported frequencies of mononuclear phagocytic cells in these tissues (bone marrow [27], blood monocytes from Ficoll-Hypaque sedimentation [4, 10], splenic monocytes or macrophages [12], and peritoneal macrophages [23, 26]). An exception to this...
observation was that the frequency of alveolar cells binding CSF-1 was much lower than the reported frequencies of alveolar macrophages in such preparations (1, 10). Indeed, many alveolar cells, which by classical morphological criteria are macrophages (N/C ≤ 1, Fig. 3d), did not bind CSF-1. (c) In all tissues examined the frequency of cells which respond to CSF-1 by forming colonies of macrophages (18) was equal to or less than the frequency of binding cells.2 Taken together, the above evidence strongly suggests that the CSF-1 receptor is restricted to undifferentiated and differentiated members of the mononuclear phagocytic cell lineage. The reason why some macrophages, i.e., the alveolar subset mentioned above, do not bind CSF-1 is unclear.

Grain counts in radioautographs indicated that the number of CSF-1 binding sites per cell varied among binding cells (Fig. 4). With the exception of the relatively homogeneous distribution for blood mononuclear cells, all tissues examined exhibited heterogeneous distributions of grain counts per cell. CSF-1 binding alveolar cells, of larger average size than binding cells from other tissues, also possessed a higher average number of receptors than cells from other tissues (Table I). In agreement with this, the frequency distribution of grain counts associated with these cells was shifted towards higher grain counts per cell compared with the distributions of CSF-1 binding cells in bone marrow and blood (Fig. 4). The observed heterogeneity among CSF-1 binding cells in tissues other than blood could be explained in a variety of ways, e.g., microenvironmental variations in CSF-1 concentration, cell cycle state, or state of differentiation.

An excellent correlation exists between the reported tissue distribution of monocytes and the tissue distribution of cells with grain counts of between 10 and 45 grains. Virtually all of the mononuclear phagocytic cells in the peripheral blood

1 In this regard, it would appear that the presence of the receptor is necessary but is not sufficient for the CSF-1 proliferative response. However, it has recently become clear that although not all CSF-1 receptor bearing cells have the capacity to proliferate, those that do not may respond to CSF-1 in other ways (9, 10).
mononuclear cell population are monocytes and 70–80% of those in bone marrow are monocytes (7, 24). CSF-1 binding blood mononuclear cells are tightly distributed (98% of binding cells have between 10 and 45 grains per cell). The major peak (71%) of the more heterogeneously distributed binding cells in bone marrow fall within this “monocyte” range. Thus it appears that the major binding cell class in blood and bone marrow is the monocyte, of which approximately two-thirds are mononuclear cells with N/C >1 and one-third cells with deeply indented nuclei (Table II) and which are relatively homogeneous in their expression of the CSF-1 receptor (~3,000 receptors per cell).

By contrast, there are very few CSF-1 binding pulmonary alveolar cells with the morphology of the CSF-1 binding blood mononuclear cells (cells with indented nuclei or small (5–10 μM) cells with N/C >1, Table II). It is significant that <5% of the CSF-1 binding cells in this population have <45 grains per cell, i.e., the alveolar cell distribution (Fig. 4 c) lacks the “monocyte” peak.

The distribution, frequencies and morphologies of CSF-1 binding cells in murine tissues suggest that the CSF-1 receptor is a specific marker of mononuclear phagocytic cells and their precursors. The results demonstrate that binding by specific growth factors may be used as a means of identifying both mature and immature cells of a differentiating cell lineage.

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