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CHEMICAL CROSSLINKING OF THE MONONUCLEAR PHAGOCYTE SPECIFIC GROWTH FACTOR CSF-1 TO ITS RECEPTOR AT THE CELL SURFACE

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The cell surface receptor for CSF-1 has been identified by affinity labeling intact mouse bone marrow-derived macrophages and intact cells of the murine J774.2 and BAC1 lines, which differ in their specific CSF-1 binding capacities and biological responses. The receptor, labeled by crosslinking to ^{125}I-CSF-1 with disuccinimidyl suberate, is a polypeptide of approximately 165,000 molecular weight, irrespective of cell type.

Colony stimulating factors are polypeptide growth factors which cause proliferation and differentiation of granulocytes and macrophages from hemopoietic precursor cells in culture (1-3). These factors comprise four subclasses based on preferential stimulation of neutrophil, eosinophil, neutrophil-macrophage, or macrophage production (reviewed in 4). The CSF-1 subclass, which has been clearly distinguished from other colony stimulating factors by specific radioimmunoassays and radioreceptor assays, stimulates mononuclear phagocytes and their precursors exclusively (4-8).

CSF-1 has been purified from mouse L-cell conditioned medium (9) and human urine (7) as a sialoglycoprotein of 45,000-76,000 molecular weight. The size heterogeneity results from substantial glycosylation of a protein core composed of a disulfide-linked dimer of approximately 28,000 molecular weight; most of the carbohydrate component can be removed without affecting biological activity (9,10).

The initial interaction of CSF-1 with its target cells is via a specific cell surface receptor which is found only on mononuclear phagocytes and their precursors and which mediates the biological activity and intracellular degradation of the growth factor (8,11-13). Developmentally early cells of

Abbreviations: BMM - murine bone marrow-derived macrophages.

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the mononuclear phagocytic series require CSF-1 for survival, proliferation and differentiation in vitro; macrophages require CSF-1 for survival in vitro, but the proliferative response of these cells appears to be dependent on tissue origin (14-16).

In order to investigate the mechanism of action of growth factors at the subcellular level, it is desirable to obtain non-primary cell types for in vitro studies. Such cell lines frequently exhibit different biological responses and receptor levels than cells in primary target tissues, to which in vitro findings must ultimately be referred. We have examined three CSF-1 receptor-bearing cell types which will be useful in studying the nature and mechanism of action of CSF-1: primary mouse bone marrow macrophages and two transformed lines, J774.2 and BAC1. The cell types differ in their requirement for and response to CSF-1 and in the extent to which they bind ^{125}I-CSF-1. Nevertheless, the CSF-1 receptor identified by SDS-gel electrophoresis and autoradiography appears as a single polypeptide of 165,000 molecular weight in each cell type.

Methods

Cell culture: C3H/HeJ murine bone marrow-derived macrophages were obtained as described (15). The BAC1 cell line, derived from adherent BALB/c x A.CA F_1 murine spleen cells by transfection with origin-defective SV-40 DNA (17) and the J774.2 cell line were kindly supplied by Dr. Betty Diamond (Albert Einstein College of Medicine, N.Y.). A rapidly growing clone of BAC1, 2F5, was used in this work. BMM and BAC1 cells were cultured in tissue culture and petri dishes, respectively as described (15). J774.2 cells were cultured in tissue culture dishes in α-medium supplemented with 10% horse serum (Gibco).

^{125}I-CSF-1 binding: Purified L cell CSF-1 was iodinated and specific binding of ^{125}I-CSF-1 to BMM, BAC1 and J774.2 cells was measured as described (8). Specific radioactivities, calculated based on percent incorporation of ^{125}I into anti-CSF-1 antibody precipitable material ranged from 3-7 x 10^5 cpm/ng. Greater than 90% of ^{125}I-CSF-1 binding could be competed by a 200-fold excess of unlabeled CSF-1.

Crosslinking of ^{125}I-CSF-1 to cells: Cells were incubated at 4° with 150 pM ^{125}I-CSF-1 in the presence or absence of 4 nM unlabeled CSF-1 and crosslinked by a modification of the method of Pilch and Czech (18). After washing with ice-cold PBS, 2 ml of cold PBS containing 0.2 mM disuccinimidyl suberate (Pierce, prepared as a 100-fold concentrate in DMSO) was added to each sample, and incubation continued at 4° for 45 min. The crosslinking reaction was quenched and samples washed once with 50 mM Tris HCl, pH 7.4. Each sample was incubated for 10 min. at 4° with dissociation buffer (70 mM sodium acetate, 50 mM NaCl, pH 4.0) to remove non-covalently attached ^{125}I-CSF-1. Samples were washed once with dissociation buffer and scraped from plates or resuspended in PBS containing 1 mM phenylmethanesulfonyl fluoride, and pelleted by centrifugation in an Eppendorf microfuge (4 min.). Pellets were

resuspended in 50 µl of 50 mM Tris-HCl pH 7.4 containing 1% SDS, 10% glycerol and pyronin-y and boiled 2 min. in the presence or absence of 5% 2-mercaptoethanol prior to electrophoresis.

Gel electrophoresis: Electrophoresis was performed according to Laemmli (19). Fixed, stained and dried gels were autoradiographed on Kodak X-OMAT R film with enhancing screen (Picker Corporation).

Results and Discussion

BMM, BAC1 and J774.2 cells are murine in origin but differ in their requirement for and responsiveness to murine CSF-1. BMM are primary cells which absolutely require CSF-1 for survival and for growth in culture (15). These cells respond pleiotropically to CSF-1 in culture; rapid changes in cell surface morphology (15, Morgan, C.J., Tynan, P.W., and Stanley, E.R., unpublished observations) are followed by changes in protein turnover and cell division (15,20). J. 774.2 cells, cloned from Balb/c/NIH reticulum cell sarcoma J774, exhibit macrophage-like characteristics (21,22). J774.2 cells bind CSF-1 (6,12) but can be maintained in continuous culture in the absence of CSF-1 and respond to its presence by very limited stimulation of DNA synthetic rate (less than two-fold versus twenty- to thirty-fold in BMM and BAC1, C.J. Morgan, unpublished observations). The BAC1 line displays macrophage markers and functions (17), is dependent upon CSF-1 for survival and proliferation in culture and is capable of continuous growth in culture in the presence of CSF-1 (17 and C.J. Morgan, unpublished observations).

As the site of initial interaction of CSF-1 with target cells (11), the CSF-1 receptor mediates different sets of responses in each cell type: no measured function to date in J774.2 cells, multiple metabolic effects plus viability and growth for a finite lifetime in BMM, and viability and continuous growth in BAC1 cells. It is of interest to determine whether the variation in observed cellular responses is due to the presence of different CSF-1 receptors or differences in the capacities of the target cells to respond to CSF-1. To this end, we have measured specific ^{125}I-CSF-1 binding and compared the apparent molecular weight of the CSF-1 receptor identified by affinity labeling in each cell type.

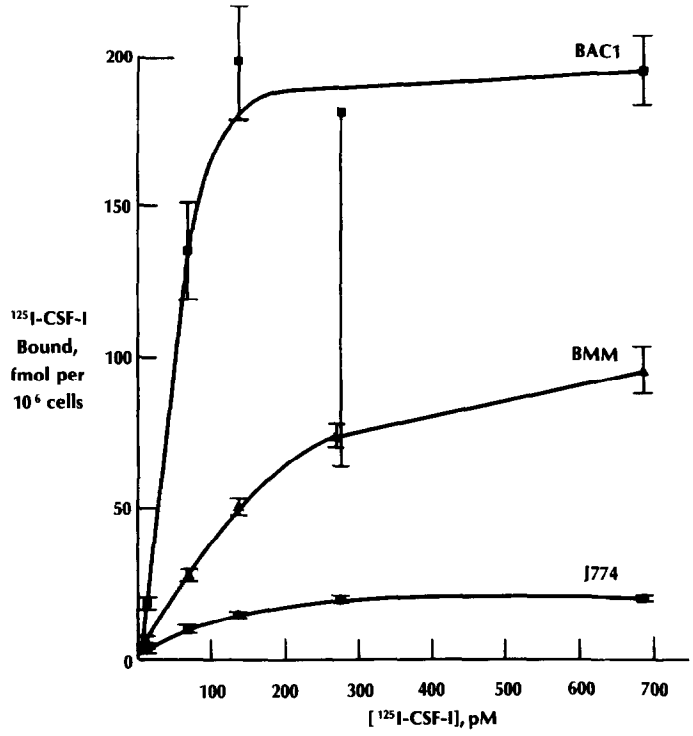

Figure 1: High affinity binding of ^{125}I-CSF-1 to intact cells. Binding to adherent BMM, BAC1 and combined adherent and non-adherent J774.2 cells at 0° was measured as described (8). Data are presented as mean ± S.D. of triplicate determinations.

Specific binding of ^{125}I-CSF-1 to BMM, BAC1 and J774.2 cells at 0° is shown in Fig. 1. While the concentration range over which ^{125}I-CSF-1 binding occurred was similar among the three cell types, the extent of ^{125}I-CSF-1 binding differed greatly; BAC1 cells bound twice as much ^{125}I-CSF-1 as BMM and ten times more than J774.2 cells.

When intact cells were chemically crosslinked with ^{125}I-CSF-1 under binding conditions, the CSF-1 receptor was specifically labeled and could be identified by SDS-electrophoresis and autoradiography (Fig. 2). This resulted in the labeling of a major band which migrated as a polypeptide of approximately 200,000 molecular weight in BMM, BAC1 and J774.2 cells (Fig. 2A). This band was not present when crosslinking was carried out in the presence of excess unlabeled CSF-1 (lanes d,f) and its mobility was independent of the reductant 2-mercaptoethanol (lanes a,b). Assuming that this band

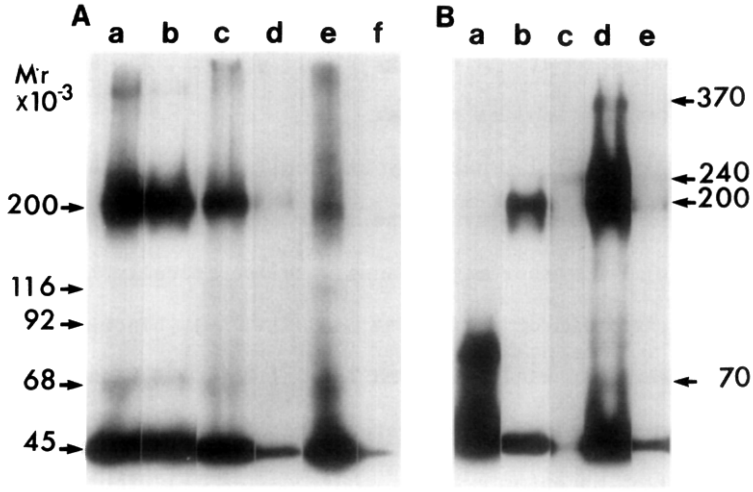

Figure 2: Affinity labeling of the CSF-1 receptor of intact cells. BMM, BAC1 and J774.2 cells were crosslinked to ^{125}I-CSF-1 as described in the methods. Panel A. Lanes a,b: BMM; lanes c,d: BAC1; lanes e,f: J774.2. Lane a: electrophoresis carried out in the absence of 2-mercaptoethanol; lanes d,f: crosslinking carried out in the presence of excess non-radioactive CSF-1. An equal amount of radioactivity was applied to each lane. Panel B. Lane a: ^{125}I-CSF-1; lanes b,c: BMM; lanes d,e: BAC1. Lanes c,e: crosslinking carried out in the presence of excess non-radioactive CSF-1. Approximately equal amounts of solubilized cell suspension were applied to lanes b-e. Unless specified, samples were reduced with 5% 2-mercaptoethanol prior to electrophoresis. Molecular weight standards were: myosin (200,000), β-galactosidase (116,000), phosphorylase b (92,000), bovine serum albumin (66,000), ovalbumin (45,000).

represented receptor crosslinked to monomeric ^{125}I-CSF-1, the estimated molecular weight of the receptor was approximately 165,000. The failure of 2-mercaptoethanol to affect the apparent molecular weight indicated that the receptor polypeptide does not exist in a disulfide-linked complex with other cellular proteins.

Other minor labeled bands migrated at approximately 370,000, 120,000 and 70,000 molecular weight. A 70,000 molecular weight band was also seen in uncrosslinked ^{125}I-CSF-1 samples (Fig. 2B, lane a). This band may represent a minor impurity (<5%) of the CSF-1 preparation, serum albumin which has become associated with ^{125}I during storage, or unreduced ^{125}I-CSF-1. The nature of the other two labeled bands is unclear. The intensity of the 120,000 molecular weight band was greater in J774.2 cells than in BAC1 or BMM. This band may have resulted from proteolysis of the 165,000 MW form. The high molecular weight band was observed consistently in BMM and BAC1 cells; whether it is

related to a lower affinity CSF-1 binding component detected in solubilized J774.2 cell membrane preparations (Yeung, Y.G., Jubinsky, P., and Stanley, E. R., manuscript in preparation) is unclear.

The increased ^{125}I-CSF-1 binding by BAC1 cells was associated with increased autoradiographic intensity of the major 200,000 molecular weight band (Fig. 2B, lanes d,e). No other major bands were specifically labeled, even though at the concentration of ^{125}I-CSF-1 used (150 pM), binding to BAC1 cells was four-fold greater than binding to BMM (Fig. 1, Fig. 2B, lanes b,d). Interestingly, a poorly resolved band at approximately 240,000 molecular weight (lane d) may represent receptor crosslinked to ^{125}I-CSF-1 dimer.

These studies indicate that the CSF-1 receptor includes a polypeptide of approximately 165,000 molecular weight which does not exist in a disulfide-linked complex in the intact membrane. These results clearly do not preclude functional non-covalent association of the CSF-1 receptor with other cellular proteins. However, preliminary studies with the purified CSF-1 receptor indicate that there are no other receptor subunits (Yeung, Y.G., Tushinski, R.J. and Stanley, E.R., unpublished observations).

The molecular weight of affinity labeled receptor is invariant in the three cells types examined, despite their different specific CSF-1 binding capacities and biological responses. The BAC1 cell line, which responds to CSF-1 similarly to primary bone marrow macrophages and has the greatest CSF-1 binding activity, should prove useful in studies of CSF-1 action at the subcellular level. The J774.2 line, despite a low level of CSF-1 binding, has been useful in purification of the CSF-1 receptor due to the relative ease and inexpense of culturing it in quantity.

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