Characterization of the Cell Surface Receptor for a Multi-lineage Colony-stimulating Factor (CSF-2α)*

Linda S. Park, Della Friend, Steven Gillis, and David L. Urda†

From the Immune Corporation, Seattle, Washington 98101

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125I-Labeled colony-stimulating factor (CSF) 2α (interleukin 3, multi-CSF, and mast cell growth factor) was used to characterize receptors specific for this lymphokine on the cell surface of the factor-dependent cell line FDC-P2. CSF-2α binding to these cells was specific and saturable. Among a panel of lymphokines and growth factors, only unlabeled CSF-2α was able to compete for the binding of 125I-labeled CSF-2α to cells. Equilibrium binding studies revealed that CSF-2α bound to 434 ± 281 receptors/cell with a $K_d$ of 8.7 ± 3.9 × 10⁶ M⁻¹. Affinity cross-linking experiments with the homobifunctional cross-linking reagents disuccinimidyl suberate, disuccinimidyl tartrate, and di-thiobis(succinimidyl propionate) produced a radiolabeled band of $M_r$ = 97,000 on intact cells and in purified cell membranes, while an additional band of $M_r$ = 138,000 was produced upon cross-linking to intact cells only. The relationship between these two bands is discussed. The results indicate that the receptor for CSF-2α on FDC-P2 cells consists at a minimum of a subunit of $M_r$ = 72,500.

CSF-2α was initially described as one of three colony-stimulating activities secreted by the murine T cell lymphoma LBRM-35A4 in response to stimulation by phytohemagglutinin (1). CSF-2α has been purified to homogeneity (2) and the NH₂ terminus of the protein sequenced (3). This analysis revealed that CSF-2α was probably identical to mast cell growth factor (4–7), IL-3 (8, 9), and P factor (10–12), all factors for which cDNA sequence or protein sequence is available.

CSF-2α is required for the proliferation of bone marrow-derived, factor-dependent cell lines such as FDC-P2 (13). It also appears to be responsible for the generation of multi-lineage specific colonies from whole mouse bone marrow which include granulocyte, macrophage, mixed granulocyte-macrophage, erythroid, megakaryocyte, and mast cell colonies (1). These biochemical and biological characteristics readily distinguish CSF-2α from other colony-stimulating factors such as granulocyte-macrophage CSF (GM-CSF (14)), granulocyte CSF (15), and macrophage CSF (CSF-1 (16)). In order to more completely understand the mechanisms behind CSF-2α action we have 125I radiolabeled CSF-2α and used this reagent to characterize receptors specific for this lymphokine on the surface of FDC-P2 cells.

EXPERIMENTAL PROCEDURES

Materials—Disuccinimidyl suberate (DSS), disuccinimidyl tartrate (DST), and di-thiobis(succinimidyl propionate) (DSP) were purchased from Pierce Chemical Co. Dimethyl sulfoxide was from Burdick and Jackson. Phenylmethylsulfonyl fluoride, pepstatin A, leupeptin, O-phenanthroline, β-g-d-glucose, and the high molecular weight standard mixture of molecular weight markers for SDS-gel electrophoresis were from Sigma. 125I as sodium iodide and the methyl-13C-labeled molecular weight markers: cytochrome c ($M_r$ = 12,300), lactoglobulin A ($M_r$ = 15,367), carbonic anhydrase ($M_r$ = 31,000), ovalbumin ($M_r$ = 45,000), bovine serum albumin ($M_r$ = 68,000), phosphorylase b ($M_r$ = 97,400), and myosin ($M_r$ = 200,000) were from New England Nuclear. Nerve growth factor, fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor were obtained from Bethesda Research Labs. Human follicle-stimulating hormone, human luteinizing hormone, human thyroid-stimulating hormone, human growth hormone, and bovine insulin were obtained from Calbiochem-Behring.

Human recombinant IL-2 was expressed in and purified from Escherichia coli and provided via a collaborative research agreement between Immunix Corp. and Hoffman-LaRoche. Mouse GM-CSF and CSF-2α were cloned from a cDNA library prepared from RNA extracted from LBRM-35A4 cells. The nucleotide sequences of these two cDNA clones were essentially identical to those reported previously (7, 9, 17). GM-CSF and CSF-2α were produced in a yeast expression system that used the prepro α factor promoter and leader sequence (18) to direct secretion of the mature forms of the two factors. Using reversed phase high performance liquid chromatography GM-CSF was purified to homogeneity and CSF-2α partially purified from yeast-conditioned medium. Human IL-1 was purified to homogeneity as previously described (19) from media conditioned by activated human macrophages.

Cell Culture—The factor-dependent cell lines FDC-P2 (13), a CSF-2α-dependent mouse bone marrow-derived line kindly provided by T. M. Dexter (Paterson Labs, Manchester, United Kingdom), and CTLL-2, an IL-2-dependent mouse T cell line (20), were maintained in culture as previously described (1). All other cells listed and referenced in Table I were grown in RPMI containing 10% fetal bovine serum, penicillin (50 units/ml), streptomycin (50 μg/ml), and glutamine (1 mM) with or without 5 × 10⁻⁵ M 2-mercaptoethanol.

CSF-2α Purification—CSF-2α was purified to homogeneity from medium conditioned by phytohemagglutinin-stimulated LBRM-35A4 cells as previously described (2) and summarized below. Protein was first sequentially precipitated from conditioned medium by the stepwise addition of ammonium sulfate to 30, 50, and finally to 80% saturation. The proteins contained in the 80% precipitate were then fractionated by cation exchange chromatography followed by anion exchange chromatography. Purification to homogeneity was achieved by reversed phase high performance liquid chromatography; first, on a C₄ Bondapak column (30 cm × 3.9 mm) equilibrated in 0.1% 3. D. Cosman, unpublished results.

V. Price, unpublished results.
4. D. Urda, unpublished results.
trifluoroacetic acid and eluted with a gradient of acetonitrile, and second on the same column but with a gradient of 1-propanol in 0.5 M acetic acid, 0.2 M pyridine (2). A third high performance liquid chromatography step was used to exchange the 1-propanol solvent for the acetonitrile solvent such that the final CSF-2a sample was contained in 100% acetonitrile.

CSF-2a activity was measured by its ability to sustain the proliferation of FDC-P2 cells (t3), a factor-dependent cell line isolated from mouse bone marrow. FDC-P2 cells die in the absence of CSF-2a. Units of CSF-2a activity were determined as the reciprocal dilution of a sample which generated 50% of maximal FDC-P2 [3H]thymidine incorporation as compared to a laboratories standard (WEHI-3b cell line conditioned medium). For example, if a sample generated 50% of maximal FDC-P2 [3H]thymidine incorporation at a dilution of 1:10, 1/10 of 100 µl (assay volume) or 10 µl was said to contain 1 unit. The sample would therefore contain 1000 + 10 or 100 units of CSF-2a activity/µl. One µg of CSF-2a corresponds to 1 x 10^7 units of activity (2).

Iodination of CSF-2a—CSF-2a was radiolabeled using the Enzymobead radioiodination reagent (Bio-Rad) essentially following the manufacturers specifications. Aliquots (2 x 10^6 units in 25 µl) of CSF-2a in acetonitrile and trifluoroacetic acid were combined with 50 µl of 0.2 M sodium phosphate, pH 7.2, and the acetonitrile was evaporated under nitrogen. Fifty µl of Enzymobead reagent, 20 µl of 1211 (2 M), and 10 µl of 2.5% β-d-glucose were added and the mixture incubated at 25 °C for 10 min. Sodium azide (20 µl of 25 mM) and sodium sulphite (10 µl of 0.5 M) were then added. After 5 min at 25 °C, iodinated CSF-2a was separated from free 1211 by chromatography on a 2-ml Sephadex G-25 column equilibrated in 0.05 M sodium phosphate, pH 7.2, containing 0.01% gelatin. Fractions containing CSF-2a were pooled, bovine serum albumin (0.01% final concentration) and sodium azide (0.02% final concentration) were added, and the pooled sample stored at 4 °C. Bioactivity of 1211-CSF-2a was determined in the FDC-P2 proliferation assay described above. Labeled CSF-2a preparations were analyzed by gel filtration chromatography on Sephadex G-75 (Pharmacia) to test for the presence of high molecular weight aggregates.

Cross-linking Plasma Membranes—Plasma membranes (100 µg) were incubated with 125I-CSF-2a (3-6 x 10^11 M) at 37 °C in 100 µl of binding medium both in the presence and absence of a 50-fold or greater molar excess of unlabeled CSF-2a. After 2 h, the membranes were harvested and washed three times in PBS by centrifugation at 12,000 g for 10 min. The final cell pellets were then resuspended in 150 µl of PBS and chilled on ice. Aliquots of DSS, DSP, or DST dissolved in dimethyl sulfoxide were then added such that the concentration of dimethyl sulfoxide in the incubation did not exceed 2%. The final concentration of cross-linker was in most cases 1 mg/ml. Control incubations were treated in an identical manner except no cross-linker was added. After 1 h at 0 °C the membranes were harvested and washed three times in PBS by centrifugation at 30,000 x g for 15 min at 4 °C. Membrane pellets were then dissolved in 40 µl of sample buffer both in the presence and absence of 5% 2-mercaptoethanol, boiled for 3 min, and subjected to electrophoresis as described below.

Cross-linking to Intact Cells—Binding assays were performed by a phthalate oil separation method described previously (21). Cells (2-10 x 10^6) were incubated with 125I-CSF-2a in RPMI-1640 containing 2% bovine serum albumin, 20 mM Hepes, containing 0.85% NaCl and 0.5 mM MgCl2, pH 7.5 (lysing medium), and then suspended to a concentration of 8 x 10^6/ml. Cells were then incubated at 37 °C in the presence of a 50-fold or greater molar excess of unlabeled CSF-2a. After 2 h the cells were centrifuged at 12,000 g for 10 min at 25 °C. The final cell pellets were then resuspended in PBS and chilled on ice. Aliquots of DSS, DSP, or DST dissolved in dimethyl sulfoxide were then added such that the concentration of dimethyl sulfoxide in the incubation did not exceed 2%. The final concentration of cross-linker was in most cases 1 mg/ml. Control incubations were treated in an identical manner except no cross-linker was added. After 1 h at 0 °C the membranes were harvested and washed three times in PBS by centrifugation at 30,000 x g for 15 min at 4 °C. Membrane pellets were then dissolved in 40 µl of sample buffer both in the presence and absence of 5% 2-mercaptoethanol, boiled for 3 min, and then subjected to electrophoresis as described below.

RESULTS AND DISCUSSION

CSF-2a, a colony-stimulating factor which is similar if not identical to IL-3 and mast cell growth factor, was radiolabeled with 125I and used to characterize the specific receptors for this lymphokine on the surface of FDC-P2 cells. Fig. 1 illustrates a autoradiograph of a typical iodinated CSF-2a preparation, where the major species after iodination had an apparent molecular weight of 24,500 on analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Based on a specific activity for CSF-2a of 10 x 10^6 units/µg protein (2), the radiolabeled preparations had estimated specific activities in the range of 1 x 10^8 cpm/mmol, and when tested in a cell proliferation assay, radiolabeled CSF-2a preparations were routinely found to retain >75% of their biological activity. Preparations of 125I-CSF-2a were stable for several weeks when stored at 4 °C in 0.05 M NaPO4, pH 7.2, containing 0.01% bovine serum albumin and 0.02% sodium azide, and
specific and nonspecific binding at both 37 and 4 °C were comparable to those reported (22; data not shown).

CSF-2α, when iodinated by the Enzymobead method was found to retain full binding affinity. Fig. 3 represents the ability of increasing concentrations of unlabeled CSF-2α to block the binding of 125I-CSF-2α to FDC-P2 cells. The inhibition constant (Kᵢ) calculated from this curve (7.80 × 10⁻⁶ ± 1.61 × 10⁻⁶ M⁻¹) was not significantly different from the Kᵢ of 125I-CSF-2α (8.7 × 10⁻⁶ ± 3.9 × 10⁻⁶ M⁻¹).

A number of continuous cell lines of mouse, rat, or human origin were examined for their ability to bind 125I-CSF-2α. As shown in Table I, none of the human lines nor the rat line tested bound any 125I-CSF-2α. Only three mouse lines, J774, P388D1 (macrophage tumor cell lines), and P815 (a mastocytoma) were found to bind detectable levels of 125I-CSF-2α, and all had less than 100 receptor sites/cell. Due to their origin, it is not surprising that these cell lines should express CSF receptors but the significance of receptor expression on

**Table I**

| Cell line     | Characteristics                  | CSF-2α bound             |
|---------------|----------------------------------|--------------------------|
| **Mouse**     |                                  |                          |
| FDC-P2        | CSF-2α dependent                 | 434 ± 281                |
| P388D1        | basophilic tumor (30)             | 0                        |
| J774          | Macrophage tumor (31)             | 90 ± 10                  |
| P815          | Mastocytoma (ascites) (32)        | 90 ± 10                  |
| P388D1-1.8    | Macrophage tumor (33)             | 50 ± 20                  |
| EL4           | T-lymphoma (34)                  | 0                        |
| Wehi 3        | Myelomonocytic tumor (35)        | 0                        |
| Wehi 256      | Monocyte tumor (36)              | 0                        |
| CTLL          | IL-2 dependent T-cell (20)       | 0                        |
| SC1           | Fibroblast (37)                  | 0                        |
| MST 7723      | B-cell lymphoma                  | 0                        |
| **Rat**       |                                  |                          |
| XC            | Fibroblast (38)                  | 0                        |
| **Human**     |                                  |                          |
| HL60          | Promyelocytic leukemia (39)      | 0                        |
| KG1           | Myelogenous leukemia (40)        | 0                        |
| U937          | Monocytic tumor (41)             | 0                        |
| K562          | Erythroleukemia (42)             | 0                        |
| BMB           | B-lymphoma                       | 0                        |
these particular lines relative to lines of similar origin tested by us, and by Palaszynski and Ihle (22), is not known. We also failed to find $^{125}$I-CSF-2α binding to the macrophage line P388D, which these investigators reported to bind $^{125}$I-IL-3 at low levels. Considering that we were able to detect binding to the macrophage tumor cell lines J774 and PU5-1.8, while these investigators detected no binding to another macrophage line they tested (RAW 264.7), it would seem that at least some, but not all, macrophage tumor lines are capable of expressing the CSF-2α receptor. Of the cell lines tested, FDC-P2 exhibited the highest number of binding sites and was therefore used in further experiments designed to examine the physical characteristics of the CSF-2α receptor.

The specificity of $^{125}$I-CSF-2α binding was examined by testing a number of purified lymphokines and other polypeptide hormones for their ability to compete with $^{125}$I-CSF-2α for binding to its receptor on FDC-P2 cells. As shown in Fig. 4, natural CSF-2α eliminated >50% of $^{125}$I-CSF-2α binding when present in 10-fold excess (column d) with up to 80% competition at higher concentrations (columns b and c). Similarly, a 20-fold excess of recombinant CSF-2α eliminated 80% of $^{125}$I-CSF-2α binding (column e). None of the other lymphokines or hormones tested, including GM-CSF, exhibited any ability to compete with $^{125}$I-CSF-2α binding to FDC-P2, even when present at concentrations that were 500–1000-fold greater (on a molar basis) than that of $^{125}$I-CSF-2α (columns f–q). Further evidence of this specificity is shown in Fig. 5. FDC-P2 cells were incubated with $^{125}$I-CSF-2α either alone (lane a) or in the presence of an excess of unlabeled natural CSF-2α (lane b), recombinant CSF-2α (lane c), recombinant GM-CSF (lane d), or recombinant IL-2 (lane e). The cells were then washed, extracted with PBS/1% Triton and the soluble fraction analyzed by SDS-PAGE. It is apparent that both natural and recombinant CSF-2α can completely block specific binding of $^{125}$I-CSF-2α to FDC-P2 cells, while recombinant GM-CSF and IL-2 have no effect. Interestingly, GM-CSF, at a concentration 500 times greater than that of the $^{125}$I-labeled CSF-2α, failed to inhibit binding of CSF-2α to cells. Other workers have suggested that GM-CSF and CSF-2α may interact with the same receptor (17). The results reported here would suggest that this is not the case. Indeed, we have recently examined the binding of $^{125}$I-labeled GM-CSF to cells and found that GM-CSF does not bind to FDC-P2 cells and, on cells that bind both factors, no evidence of receptor cross-reactivity is detected. These observations do not rule out, however, the possibility that on cells expressing both receptors, the binding of one ligand will result in the down-regulation of receptors for the second ligand.

The CSF-2α receptor is present on FDC-P2 cells in low abundance. One means of characterizing such receptors is by affinity labeling (29). Radiolabeled CSF-2α was bound to cells as described above. Then, the cells were washed and the bifunctional cross-linking reagents DSS, DSP, or DST (1 mg/ml) were added in order to covalently cross-link the $^{125}$I-CSF-2α to its plasma membrane receptor. Cells treated in such a manner were then extracted with PBS/1% Triton and the soluble fraction analyzed by SDS-PAGE (Fig. 6). Under both reducing (panel A) and nonreducing (panel B) conditions two major cross-linked species were evident with M, of approximately 138,000 and 97,000. In addition, some cross-linked material of very high molecular weight was collected at the top of the separating gel. Both the 138,000 and 97,000 species were present whether cross-linking was done with DSS (lanes c and d), DSP (lanes e and f), or DST (lanes g and h). The results of densitometric analysis of 10 different cross-linking experiments are summarized in Table II and suggest that the two bands were present in approximately equal amounts. Controls showed no cross-linked species were found in the absence of cross-linker (lanes a and b) or in samples containing excess unlabeled CSF-2α (lanes h, d, f, and h). In addition, as expected, cells cross-linked with the disulfide containing cross-linker DSP, showed no cross-linked species when run on SDS-PAGE under reducing conditions (panel A, lanes e and f).

In order to examine the nature and possible relationship of these cross-linked species, several parameters in the cross-

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**Fig. 5. Characterization of $^{125}$I-CSF-2α binding to FDC-P2 cells by SDS-polyacrylamide gel electrophoresis.** FDC-P2 cells (8 x 10⁶) were incubated with $^{125}$I-CSF-2α (4 x 10⁻¹¹ M) and the following unlabeled compounds at the concentrations indicated: a, none; b, CSF-2α, 2.5 x 10⁻⁸ M; c, recombinant CSF-2α, 5.3 x 10⁻⁸ M; d, recombinant mouse GM-CSF, 2.5 x 10⁻⁸ M; e, recombinant human IL-2, 2.3 x 10⁻⁷ M. Incubation was for 2 h at 37 °C after which cells were harvested, washed, and extracted with PBS/1% Triton containing 2 mM PMSF as described under "Experimental Procedures." Aliquots corresponding to 1.5 x 10⁶ cells were dried under vacuum, dissolved in sample buffer containing 2% SDS and 5% 2-mercaptoethanol, boiled for 3 min, and subjected to SDS-polyacrylamide gel electrophoresis on a linear 5–15% gradient gel.

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5 L. S. Park and D. L. Urdal, unpublished results.
Fig. 6. $^{125}$I-CSF-2α cross-linking to intact FDC-P2 cells.

FDC-P2 cells (8 x 10^6) were incubated with $^{125}$I-CSF-2α (4 x 10^{-9} M) both in the presence and absence of unlabeled CSF-2α (2.5 x 10^{-9} M). Incubation was for 2 h at 37 °C. Cells were then harvested, washed, cross-linked for 1 h at 4 °C with DSS, DST, or DSP (1 mg/ml) and extracted with PBS/1% Triton containing 2 mM PMSF as described under "Experimental Procedures." Aliquots corresponding to 1.5 x 10^6 cells were dried under vacuum, dissolved and boiled for 3 min in sample buffer containing 2% SDS, both with (panel A) and without (panel B) 5% 2-mercaptoethanol, and subjected to electrophoresis on a 8% polyacrylamide gel. Panels A and B contain identical aliquots of samples cross-linked with: a and b, no cross-linker; c and d, 1 mg/ml DSS; e and f, 1 mg/ml DSP; g and h, 1 mg/ml DST. Lanes b, d, f, and h correspond to samples which contained unlabeled CSF-2α during incubation of $^{125}$I-CSF-2α with FDC-P2 cells. The abundance of the M_r = 97,000 species relative to the M_r = 138,000 species was determined by scanning densitometry. In panel A: lane c, 23% was the 97,000 band; lane g, 39%; in panel B: lane c, 48% was the 97,000 band; lane e, 56%; lane g, 60%.

Table II

Relative abundance of $^{125}$I-CSF-2α cross-linked species

Cross-linking experiments were performed as described in the legend to Fig. 6 and the relative abundance of the M_r = 138,000 and 97,000 species present on autoradiographs assessed by densitometric scanning. Data are expressed as the percentage of cross-linked material present as the M_r = 97,000 species averaged from 10 separate experiments.

| Cross-linker | SDS-PAGE conditions |
|--------------|---------------------|
|              | Reducing            | Nonreducing        |
| 1 mg/ml DSS  | 46 ± 23             | 41 ± 7             |
| DSP          | 50 ± 8              |                    |
| DST          | 46 ± 14             | 51 ± 19            |

linking experiments were varied. First, the effect of time of incubation and concentration of cross-linker on the presence of the cross-linked species was examined. In the presence of 1 mg/ml DSS, approximately equal ratios of the two lower molecular weight cross-linked species appeared within 5 min and no subsequent change was observed with further time of incubation (data not shown). When the concentration of DSS was varied from 0.03 to 2.0 mg/ml, with an incubation time of 66 min, there was no increase in the abundance of the 138,000 M_r species relative to the 97,000 M_r species. With increasing concentration of cross-linker, there was, however, an increase in the abundance of the very high molecular weight material present at the top of the separating gel suggesting that this material probably represents aggregates due to the high concentration of cross-linker. The nature and relationship of the two lower cross-linked species was not readily apparent from these experiments. Further experiments showed that identical results were obtained if $^{125}$I-CSF-2α binding to FDC-P2 cells was done at 4 °C rather than 37 °C. In addition, incubation of $^{125}$I-CSF-2α bound FDC-P2 cells in 25 mM EDTA for 30 min at 4 °C prior to cross-linking with DSS did not effect the appearance of either of the cross-linked species, suggesting that neither of the cross-linked species was a loosely bound peripheral membrane protein. The possibility that the 97,000 M_r species was a proteolytic cleavage product of the 138,000 M_r species was addressed by including in the extraction buffer a mixture of protease inhibitors which contained FMSF (2 mM), peptatin A (10 μM), leupeptin (10 μM), O-phenanthroline (2 mM), and EGTA (2 mM). Both the 138,000 and 97,000 M_r species were found to be present in similar amounts under all conditions examined.

In addition to the cross-linking of $^{125}$I-CSF-2α to its receptor on intact FDC-P2 cells, we also examined cross-linking to FDC-P2 plasma membrane preparations. Conditions for $^{125}$I-CSF-2α binding and cross-linking to plasma membranes were identical to those used in experiments with intact cells, except that following the cross-linking step, washed membranes were dissolved directly in SDS containing sample buffer rather than extracted with PBS/1% Triton. Fig. 7 shows the results of cross-linking $^{125}$I-CSF-2α to FDC-P2 plasma membrane preparations with DSS (lanes a and b) or DST (lanes c and d), and analyzing the samples on SDS-PAGE under reducing conditions. In both cases a single cross-linked species of approximately M_r = 97,000 was present, with no evidence of the 138,000 M_r species seen after cross-linking to intact cells. No cross-linked species were found in the absence of cross-linker (lanes e and f) or in samples containing excess unlabeled species.
CSF-2α (lanes b, d, and f). Identical results were obtained when samples were run on SDS-PAGE under nonreducing conditions. Furthermore, when plasma membranes were prepared from FDC-P2 cells in the presence of the protease inhibitor mixture described above and cross-linking experiments performed, only the 97,000 Mₐ species was detected. This band most likely represents a plasma membrane protein of Mₐ 72,500 that is covalently linked to ³⁵S-CSF-2α (Mₐ = 24,500). The possibility that the 97,000 band is actually a proteolytic cleavage product of the 138,000 Mₐ band is possible but unlikely. A panel of protease inhibitors had no effect on the appearance of either species in whole cells or plasma membrane preparations. This would then suggest that the 138,000 Mₐ band represents either the Mₐ = 97,000 receptor complex coupled to a third protein of Mₐ = 41,000 or a protein of Mₐ = 135,000 that is directly coupled to CSF-2α. While we cannot yet distinguish between these two possibilities, it is apparent that during the preparation of plasma membranes, the positioning of this putative protein(s), which allows it to be either directly or indirectly cross-linked to CSF-2α, is disrupted. Whether this protein might be part of a specific CSF-2α receptor complex, or is simply a closely associated plasma membrane or cytoskeletal protein remains to be determined.

Using CSF-2α radiolabeled to high specific activity with ³⁵S, we have investigated the nature of the receptor for CSF-2α on FDC-P2 cells through the use of bifunctional cross-linking reagents. The data detailed herein establish the existence of CSF-2α receptors on FDC-P2 cells and characterize for the first time the molecular nature of the receptor.

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