Dexamethasone Increases Expression of Mannose Receptors and Decreases Extracellular Lysosomal Enzyme Accumulation in Macrophages*

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Macrophages express a mannose-specific pinocytosis receptor that binds and internalizes lysosomal hydrolases. Treatment of rat bone marrow-derived macrophages with dexamethasone resulted in a concentration- and time-dependent increase in mannose-receptor activity. The dexamethasone effect was maximal at 24 h. Half-maximal effects were observed at a dexamethasone concentration of 2.5 × 10⁻⁸ M. With ¹³C-β-glucuronidase as ligand, a 2.5-fold increase in uptake rate was observed in dexamethasone-treated cells, with no change in Kₘ uptake (2.5 × 10⁻⁷ M β-glucuronidase). Cell surface binding (4 °C) was elevated 2.6-fold following dexamethasone treatment. The increase in ligand binding appeared to be due to an increase in number of sites with no change in affinity. Cycloheximide suppressed the dexamethasone-mediated rise in receptor number, while cycloheximide alone had little effect on receptor activity over 16 h. These results suggest that dexamethasone stimulates synthesis of mannose receptors in macrophages. Extracellular accumulation of hexosaminidase was sharply reduced by dexamethasone treatment, and corresponded with the rise in mannose-receptor activity. Extracellular levels of hexosaminidase from untreated macrophages were modestly increased by the presence of mannose, while the extracellular activity from dexamethasone-treated cells was increased significantly by mannose. Extracellular hexosaminidase, released from zymosan-treated macrophages, was dramatically reduced by dexamethasone pretreatment. Enzyme released from zymosan-stimulated macrophages was efficiently endocytosed by dexamethasone-treated cells in co-culture experiments, and this endocytosis was blocked by the addition of mannan. These results suggest that the mannose receptor of macrophages may play a role in regulating extracellular levels of lysosomal enzymes via a secretion-recapture mechanism.

Lysozymal function in macrophages has been widely studied principally because these cells are highly endocytic. Moreover, they can be stimulated to secrete large quantities of lysosomal hydrolases (1). Macrophages are known to express at least two receptors which could control the movement of hydrolases within and between cells (2, 3). Results from our laboratory and others over the past several years have shown that one of these receptors recognizes mannose-containing glycoconjugates; lysosomal enzymes are among the ligands which bind with high affinity (3, 4). From binding and uptake studies on a variety of macrophages, it has been shown that the mannose receptor binds ligands at the cell surface and that receptor-ligand complexes are rapidly internalized. Once inside prelysosomal vesicles, receptor-ligand complexes dissociate under the influence of a reduced pH and the ligand is subsequently transported to lysosomes. The receptor, having been separated from the ligand, recycles to the cell surface (5, 6). Thus, macrophages have an inordinate capacity to remove lysosomal hydrolases from their environment.

Mannose-receptor activity is not present on monocytes (7) and is poorly expressed on bone marrow cells in early stages of macrophage differentiation. Additionally, expression is low in macrophages that have been activated to a tumoricidal state (8, 9). Physiologic studies indicate that the mannose receptor may play a role in the phagocytosis of zymosan (10, 11). Thus, the mannose receptor may participate in a variety of physiologic functions involving both up-regulation and down-regulation of receptor expression.

Macrophages infiltrate areas of inflammation and secrete lysosomal hydrolases into the extracellular space (12). Glucocorticoids are used extensively as anti-inflammatory agents. These drugs interact directly with specific glucocorticoid receptors and dramatically alter the biochemical properties of inflammatory macrophages (13, 14). Given the fact that the mannose receptor is closely regulated, our initial hypothesis was that anti-inflammatory steroids may be important modulators of receptor activity. In the present study, we have investigated the effect of dexamethasone treatment on the mannose-receptor activity of rat bone marrow-derived macrophages. Moreover, we have explored the possible role of the mannose receptor in the regulation of extracellular and intracellular levels of lysosomal enzymes in macrophages.

EXPERIMENTAL PROCEDURES AND RESULTS

1 Portions of this paper (including "Experimental Procedures," "Results," Tables I-V, and Figs. 1-6) are presented in miniprint at the end of this paper. The abbreviations used are: Dex, dexamethasone; BSA, bovine serum albumin; Man-BSA, mannosylated bovine serum albumin; HBSS, Hanks' balanced salt solution; MEM, minimal essential medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TES, 2-(2-hydroxyethyl)-1-bis(hydroxymethyl)aminomethanesulfonic acid; PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry.

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DISCUSSION

Macrophages have been shown to play a major role in host responses at sites of inflammation. In response to various stimuli, macrophages secrete large quantities of lysosomal hydrolases. Should these hydrolases accumulate in the extracellular environment they may contribute to local tissue damage. In this regard, glucocorticoids have been used as anti-inflammatory agents and, in the presence of these drugs, many effects on the mononuclear phagocyte system have been observed. In vivo, monocytopenia occurs (21), monocyte response to chemotactic factors is reduced (22), and macrophages fail to respond to activating agents (23). Welb et al. (13, 14) have studied in detail the effects of glucocorticoids on macrophages in vitro. They found specific glucocorticoid receptors on monocytes and macrophages which appeared to mediate the subsequent biochemical effects. Elastase, collagenase, and plasminogen activator secretion were inhibited following dexamethasone or cortisol treatment.

Macrophages have on their cell surface a number of endocytosis receptors which mediate the uptake of complement- or IgG-containing ligands, mannosylated glycoconjugates (such as lysosomal enzymes), and α2-macroglobulin-trypsin complexes. All of these receptors play a specific role in removing ligands from the extracellular space, and all may be important in the regulation of macrophage function during immune responses. Very little information is available concerning the effect of glucocorticoids on the function of these receptors. In the present study, we have described in detail the effects of dexamethasone on the macrophage receptor which mediates uptake of mannose-containing glycoconjugates. Dexamethasone dramatically increases the number of cell surface mannose receptors, without altering the affinity of the receptor for the ligand or the rate at which ligands are internalized (Table II). The response to dexamethasone is time dependent with the maximum increase in uptake achieved after 18–24 h of treatment. Following removal of the drug, uptake rates fall slowly; at 24 h, uptake is still elevated to 65% over the control level. This is in close agreement with the time course of dexamethasone-induced inhibition of elastase, collagenase, and plasminogen activator secretion from macrophages (13, 14); effects were maximal at 24 h and were reversed during a similar time following removal of the drug.

From studies with cycloheximide, dexamethasone appears to produce the increase in mannose receptors through an increase in receptor biosynthesis. In macrophages treated with cycloheximide (0.5 μg/ml for 18 h), protein synthesis is nearly completely blocked, although uptake of β-glucuronidase is unaffected during this period. In the presence of cycloheximide the dexamethasone-induced increase in uptake is reduced from 250% of control to approximately 140%. The lack of an effect by cycloheximide on ligand uptake over 18 h indicates that the receptor must have a long half-life in macrophages. Similar observations have been made with other endocytosis receptors (e.g. the hepatocyte galactose receptor half-life is approximately 88 h (24)). Since the dexamethasone effect is significantly reduced by cycloheximide, it is likely that a substantial portion of the increase in receptor activity is due to new receptor synthesis.

Although little information is available on glucocorticoid effects on other endocytic receptors, several studies have demonstrated that at least two hormone receptors are similarly affected by dexamethasone treatment. Baker et al. (25) have shown that dexamethasone causes an increase in 125I-labeled epidermal growth factor binding to human fibroblasts with maximal stimulation occurring at dexamethasone concentrations of 100–250 ng/ml following 24 h of treatment. These workers suggested that, rather than an increase in total receptor number, their observations were consistent with a change in affinity or cooperativity. Dexamethasone has also been shown to increase insulin binding to cultured rat hepatocytes (26) and 3T3 cells (27). In the latter study, dexamethasone caused an increase in receptor number, without affecting the affinity. However, this effect did not appear to be due to an increase in receptor biosynthesis, but rather to a decrease in the rate of receptor degradation. In hepatocytes, on the other hand, insulin receptor levels were increased by a dexamethasone-induced stimulation of receptor biosynthesis. The effects of dexamethasone on mannose receptors in macrophages appear similar to the latter.

The physiologic significance of the increase in macrophage mannose receptors mediated by dexamethasone remains uncertain. One possible function of this receptor may be to control extracellular levels of lysosomal enzymes. The decrease in extracellular lysosomal hydrolase accumulation observed with dexamethasone appears to coincide with the elevation in mannose-receptor expression. The mechanism by which dexamethasone diverts newly synthesized lysosomal hydrolases from an apparent constitutive secretory pathway to a pathway which results in intracellular accumulation is not at all clear. The mannose receptor may play a significant role in regulating the net movement of lysosomal hydrolases within macrophages, but firm evidence is lacking. More likely, the mannose receptor plays a role by regulating reuptake of secreted lysosomal hydrolases. This is supported by the data in Table IV which indicate that mannose increases extracellular hexosaminidase levels, presumably by blocking reuptake via the mannose receptor. To examine the possible role of secretion-recapture via the mannose receptor in the regulation of extracellular enzyme accumulation, coculture experiments were carried out. In these studies, one population of macrophages was stimulated to secrete pre-endocytosed 125I-β-glucuronidase by incubation with zymosan. A second population of cells was then added following removal of the zymosan. The second population consisted of macrophages either untreated or pretreated with dexamethasone. The addition of untreated macrophages reduced the extracellular accumulation of secreted lysosomal hydrolases, with an even more pronounced decrease following addition of dexamethasone-treated cells. That this reduction is mannose-receptor mediated is strongly suggested by the observation that it was reversed by yeast mannose and because addition of mannose and U937, cells which are mannose-receptor negative, had no effect.

In summary, results from the present study indicate that dexamethasone reduces extracellular lysosomal enzyme accumulation in macrophages without affecting apparent synthesis and that this effect appears due in part to an elevation of mannose receptor activity. Dexamethasone increases receptor levels apparently by directly stimulating new receptor synthesis. The secretion-recapture hypothesis, first put forward by Hickman and Neufeld (28) to account for the exaggerated lysosomal enzyme secretion in I-cell fibroblasts, now appears to be a poor model for lysosomal transport in normal fibroblasts. Macrophages, on the other hand, would appear to more precisely fit the secretion-recapture model. On the basis of the present data, secretion-recapture of lysosomal hydrolases mediated by the mannose receptor may be a major factor...
in the regulation of extracellular and intracellular lysosomal enzyme levels in macrophages.

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SUPPLEMENTARY MATERIAL

Effect of Dexamethasone on Macrophage Mannose Receptors

by

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162

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Effect of Dexamethasone on Macrophage Mannose Receptors

Table II

| Time (24 hours) | Uptake (log nmoles/mg cell protein) | % Control | LIGAND ADDED (µg/µl) |
|----------------|-----------------------------------|-----------|----------------------|
| 0              | 1.83                              | 100       | 0                    |
| 24/24          | 4.84                              | 255       | 255                  |
| 240/24         | 2.82                              | 54        | 255                  |

Effect of Dex on the Binding of 125I-Man to binding sites on the ligand-binding assay. Uptake of 125I-Man was decreased by 50% due to Dex treatment, as measured by a decrease in the log nmoles/mg cell protein. The % control indicates the decrease in uptake compared to control cells.

Table III

| Addition        | % Control | LIGAND ADDED (µg/µl) |
|-----------------|-----------|----------------------|
| None            | 100       | 0                    |
| dexamethasone   | 5.13      | 255                  |
| cytosolamine    | 1.83      | 1.83                  |

Effect of Dex on the Binding of 125I-Man to binding sites on the ligand-binding assay. Uptake of 125I-Man was decreased by 50% due to Dex treatment, as measured by a decrease in the log nmoles/mg cell protein. The % control indicates the decrease in uptake compared to control cells.

Fig. 3. Effect of Dex on the Binding of 125I-Man to binding sites on the ligand-binding assay. Uptake of 125I-Man was decreased by 50% due to Dex treatment, as measured by a decrease in the log nmoles/mg cell protein. The % control indicates the decrease in uptake compared to control cells.
Effect of Dexamethasone on Macrophage Mannose Receptors

![Graph showing extracellular mannose accumulation](image)

**Table IV**

| Treatment | Hexosaminidase Activity (units/mg protein) |
|-----------|------------------------------------------|
| None      | 416.5 ± 35.3                             |
| Dexameth. | 524.4 ± 15.1                             |
| Dex        | 217 ± 22.5                               |
| None      | 437 ± 10.3                               |

**Table V**

| Hexosaminidase (units/mg protein) Diffusible Dexameth. | Dexameth. |
|--------------------------------------------------------|-----------|
| None                                                   | 20.7 ± 0.7 |
| Untreated macrophages                                   | 15.0 ± 1.5 |
| Dexameth. treated macrophages                           | 15.0 ± 1.5 |
| Dexameth. treated macrophages                           | 16.7 ± 1.2 |

a) Distinct by means of the non-linear reduction in extracellular mannose accumulation. As shown in Figure 2, the treatment for 20 hours resulted in a decrease in extracellular mannose accumulation to 10% of the control level. The number of measured macrophages in the macrophage receptor to normal and dexameth. cells, however, did not differ significantly. The results in Figure 3 show an increased extracellular mannose levels in control cells and a significant decrease in dexameth. treated cells. This suggests that the decrease in extracellular mannose accumulation is due to the increased mannose receptor activity in dexameth. treated cells, increasing the level of mannose in the extracellular fluid. The results are from 3 separate experiments.