The activities of lysosomal enzymes, such as lysozyme, acid protease, and \( \beta \)-glucuronidase, were induced with glucocorticoids during differentiation of cultured mouse myeloid leukemia line cells (M1). The induction of lysozyme activity accompanied by phagocytosis in M1 cells was most pronounced in these lysosomal enzymes. Although lysozyme activity could not be detected in undifferentiated cells, it showed a marked increase from the second day of incubation of M1 cells with dexamethasone. On the 5th day of incubation, the activity was about one-half that of mouse peritoneal macrophages.

There was a competitive interaction among different steroids on induction of lysozyme activity in M1 cells. 11\( \beta \)-Hydroxyprogesterone and 11-deoxycortisol could not induce the maximal level of the activity, and cortisone and fluoxymesterone were not active in inducing the enzyme activity. However, these steroids inhibited the induction of enzyme activity with dexamethasone, prednisolone, or hydrocortisone. Induction of lysozyme activity and phagocytic activity of M1 cells with dexamethasone was inhibited by 5-bromodeoxyuridine or puromycin at a concentration that did not interfere with the growth of cells. Differentiation could not be induced in DR-6 cells isolated from M1 cells even in a high concentration of dexamethasone. Neither lysozyme activity nor other lysosomal enzyme activities could be induced in DR-6 cells with dexamethasone.

These results indicate that glucocorticoids are capable of inducing some lysosomal enzyme activities as well as morphological and functional differentiation in M1 cells, and among these enzymes, lysozyme activity is a fairly suitable biochemical marker for the differentiation of M1 cells.

M1 cells are myeloid leukemia cells established from an SL strain mouse that can be induced to differentiate into forms that are functionally and morphologically similar to macrophages and granulocytes with conditioned media from various cell sources,\(^4,5,12,15\) serum from mice injected with endotoxin,\(^6\) ascitic fluids of various tumors,\(^10\) or glucocorticoids.\(^8,18\) In addition to morphological changes, phagocytic and locomotive activities were employed to monitor functional properties of the differentiated cells. Recently it was found that some lysosomal enzyme activities were induced during differentiation of M1 cells with a conditioned medium from cultured mouse embryo cells.\(^20\) This biochemical information may help to understand molecular mechanisms of differentiation in M1 cells.

We previously reported that M1 cells were induced to differentiate into cytologically macrophage- and granulocyte-like cells with various glucocorticoids.\(^8\) In order to understand the mechanisms of differentiation of
M1 cells at the molecular level, the induction of lysosomal enzyme activities, a typical biochemical marker of macrophages and granulocytes, in M1 cells with glucocorticoids was examined during differentiation of the cells to macrophage- and granulocyte-like cells.

**Materials and Methods**

**Chemicals** 11-Epicortisol and 5α-dihydrocortisol were obtained from Makor Chemicals Ltd., Jerusalem, and all other steroids were purchased from Sigma Chemical Co., U.S.A. Stock solutions were prepared in absolute ethanol at a concentration of 2×10⁻⁴M. Enzyme substrates (Micrococcus leisodykticus, hemoglobin, p-nitrophenyl-β-D-glucuronide, and p-nitrophenyl phosphate) were obtained from Sigma Chemical Co., U.S.A. Metabolic inhibitors, such as 5-fluorodeoxyuridine (FdU), 5-bromodeoxyuridine (BrdU), and puromycin, were also purchased from Sigma Chemical Co., U.S.A.

**Cell Line and Cell Culture** The cells used in the present study were a clone of M1 cell line, DS-4 cells, isolated from a spontaneous myeloid leukemia of an SL mouse.¹¹ The cells were cultured in suspension, in 6-cm Falcon plastic dishes, in Eagle’s minimum essential medium with twice the normal concentrations of amino acids and vitamins, and supplemented with 10% heat-inactivated calf serum and 160 µg/ml of kanamycin sulfate. The cells were cultured at 37° in a humidified atmosphere of 5% CO₂.

A subline, DR-6, derived from M1 cells, was defective in induction of phagocytosis and locomotive activity even in a high concentration of dexamethasone.

**Mouse Peritoneal Macrophages** On the 3rd day after intraperitoneal injection of 2.5 ml of 5% starch and protease peptone solution, mice were sacrificed and macrophages in their peritoneal cavity were harvested by rinsing out three times with phosphate-buffered saline (PBS). After incubation at 37° for 40 min, peritoneal macrophages were collected by scraping them off with a rubber policeman.

**Conditioned Medium** Secondary-passage cells from embryos of SL strain mice were inoculated at a cell density of 5×10⁵ cells in 5 ml of the medium described above in 6-cm Falcon plastic dishes. The cultured medium (ECM) was collected after 4 days by centrifugation and stored at −20°.

**Assay of Phagocytic Activity** The phagocytic activity was assayed by the method of Ichikawa.¹¹,¹² The cultured cells were collected by centrifugation, resuspended in serum-free medium, and incubated with addition of polystyrene latex particles (2 µl/ml, Dow Chemical Co., Indianapolis, U.S.A.) at 37° for 4 hr. The cells were collected and washed vigorously four times with PBS. The percentage of phagocytic cells among at least 300 viable cells was calculated.

**Preparation of Cell Lysate** Cells collected from cultured M1 cells with various treatments and from peritoneal macrophages in mice were washed twice with chilled PBS and then with 0.34M sucrose solution adjusted to pH 7.4 with NaHCO₃. The packed cells were resuspended to a cell density of 1×10⁷ cells/ml of 0.34M sucrose solution, frozen rapidly to −20°, and used as an enzyme solution within 1 week.

**Enzyme Assays** Lysozyme: Lysozyme activity was determined by the modified lysoplate method of Osserman and Lawlor, in which the lysoplate contained 1% Difco agar, M/15 sodium phosphate buffer (pH 6.6), 0.05M NaCl, and 0.5 mg/ml heat-killed bacterial cells of Micrococcus leisodykticus. After a 24-hr incubation at 27°, the diameter of the cleared zone due to lysozyme action on the organisms was measured. One unit was defined as equivalent to 1 µg of egg white lysozyme under this condition. In this lysoplate method, the optimal condition for the egg white lysozyme activity was different from that of lysozyme activity induced in M1 cells. Egg white lysozyme activity was optimal at pH 7.5 in the presence of 0.1M NaCl and 1 µg of egg white lysozyme was 2.94 units under this optimal condition.

Acid Protease: Enzyme activity was assayed as described previously.¹⁹ The reaction mixture consisted of 10 mg/ml of denatured hemoglobin, 0.1% (v/v) Triton X-100, and 0.1M sodium citrate buffer of pH 3.0. The mixture was preincubated at 37° for 30 min, and the reaction was started by the addition of cell lysate to give a final volume of 1 ml. Incubation was continued at 37° for 120 min. The reaction was terminated by the addition of 1 ml of ice-chilled 8.2% trichloroacetic acid and the mixture was filtered. The amount of non-precipitable protein hydrolysate in the filtrate was determined by the method of Lowry et al.¹⁹

β-Glucuronidase: The reaction mixture contained 0.1M sodium acetate buffer (pH 4.5), 0.1% (v/v) Triton X-100, 2.7mM p-nitrophenyl β-D-glucuronide, and the enzyme solution in a total volume of 1.1 ml. The reaction mixture was incubated at 37° for 60 min. The reaction was stopped by the addition of 2 ml of 0.5M NaOH, and absorbance of p-nitrophenol was determined at 405 nm. The enzyme activity was calculated.
LYSOSOMAL ENZYMES AND DIFFERENTIATION

from a calibration curve prepared with p-nitrophenol. The reaction mixture contained 38 mM sodium citrate buffer (pH 4.8), 0.1% (v/v) Triton X-100, 4.2 mM p-nitrophenyl phosphate, and the enzyme solution in a total volume of 1.2 ml. The incubation was allowed to proceed for 15 min at 37° and then the reaction was stopped by the addition of 2 ml of 0.1 M NaOH. The absorbance of p-nitrophenol was determined at 410 nm for calculation of the enzyme activity as described previously.

Acid phosphatase: The reaction mixture contained 38 mM sodium citrate buffer (pH 4.8), 0.1% (v/v) Triton X-100, 4.2 mM p-nitrophenyl phosphate, and the enzyme solution in a total volume of 1.2 ml. The incubation was allowed to proceed for 15 min at 37° and then the reaction was stopped by the addition of 2 ml of 0.1 M NaOH. The absorbance of p-nitrophenol was determined at 410 nm for calculation of the enzyme activity as described previously.

Acid DNase and Acid RNase: Activities of acid DNase and acid RNase in M1 cells were assayed as described previously.

RESULTS
Induction of Phagocytosis, and Lysozyme and Acid Protease Activity in M1 Cells with Glucocorticoids

Several lysosomal enzyme activities were determined during differentiation of M1 cells with glucocorticoids. At the onset, the time course of induction of lysozyme activity in M1 cells after the addition of various concentrations of dexamethasone was pursued (Fig. 1). The activity was detected from the 2nd day after the addition of dexamethasone and increased progressively with higher dexamethasone concentrations over the range of $5 \times 10^{-9}$ to $5 \times 10^{-6} M$. On the other hand, lysozyme activity in the untreated cells was not detectable throughout the incubation period. The time course of induction of enzyme activity with prednisolone and hydrocortisone followed a similar pattern.

Lysozyme activity induced with dexamethasone exhibited optimal pH at 6.6 which was different from the value of pH 7.5 for egg white lysozyme using this method of enzyme assay. The effect of NaCl on this enzyme activity was also examined. On the addition of 0.05 M NaCl to the reaction mixture with the enzyme, the activity increased to as much as 65% of that in the absence of NaCl. However, the activity was reduced to about 24% in the presence of 0.1 M NaCl, which was optimal for egg white lysozyme.

Induction of phagocytosis, and lysozyme and acid protease activity in M1 cells with various steroids was examined as shown in Table I. Although both phagocytosis and lysozyme activity were similarly induced with steroids with glucocorticoid action, the induction of acid protease activity was not so significant (Table I). We previously reported that there was a competitive interaction among different steroids on the induction of phagocytic activity in M1 cells. The effect of competitive interaction among different steroids on the induction of lysozyme activity was then studied. Lysozyme activities in M1 cells were determined after incubation of the cells with $5 \times 10^{-6} M$ dexamethasone in the presence of various steroids at $5 \times 10^{-6} M$. As shown in Table II, dexamethasone, prednisolone, and hydrocortisone were optimal inducers which induced the enzyme activity to a maximal level. 11β-Hydroxyprogesterone and 11-deoxycortisol were rather moderate inducers of the enzyme activity and could not induce the maximal level even at a high

![Fig. 1. Time-course of induction of lysozyme activity in M1 cells as a function of dexamethasone concentration](image)

M1 cells (2 x 10⁶ cells/ml) were incubated at steroid concentrations of 0 (○), $5 \times 10^{-8} M$ (●), $5 \times 10^{-9} M$ (▲), $5 \times 10^{-7} M$ (□), and $5 \times 10^{-6} M$ (■). Samples were removed at the time indicated and assayed for lysozyme activity.
concentrations but competitively inhibited induction of the enzyme activity by the optimal inducers. Cortisone and fluoxymesterone could not induce the enzyme activity, but competitively inhibited induction of the enzyme activity by the optimal inducers, although the degree of inhibition was less than that of moderate inducers. 5α,3β-Tetrahydrocortisol and 11-epicortisol were inactive steroids that neither induced nor inhibited the enzyme activity.

### Some Properties of Acid Protease in M1 Cells Induced with Dexamethasone

Since induction of acid protease and phagocytosis in M1 cells with various steroids is related (Table I), kinetics of the induction of enzyme activity and some properties of the induced enzyme activity were investigated. The time course of the induction of acid protease activity in M1 cells with dexamethasone is indicated in Fig. 2. The activity was significantly enhanced on the 1st day of

<table>
<thead>
<tr>
<th>Steroids (10−6M)</th>
<th>Phagocytosis (%)</th>
<th>Lysozyme (units/mg protein)</th>
<th>Acid protease (μg hemoglobin/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.4</td>
<td>&lt;0.1</td>
<td>19.9</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>41.1</td>
<td>33.5</td>
<td>35.9</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>30.0</td>
<td>25.6</td>
<td>—</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>29.3</td>
<td>20.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>22.9</td>
<td>10.9</td>
<td>30.2</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>26.2</td>
<td>11.2</td>
<td>29.4</td>
</tr>
<tr>
<td>11-Deoxycorticosterone</td>
<td>21.0</td>
<td>8.8</td>
<td>—</td>
</tr>
<tr>
<td>11β-Hydroxyprogesterone</td>
<td>20.8</td>
<td>6.8</td>
<td>—</td>
</tr>
<tr>
<td>5α-Dihydrocortisol</td>
<td>13.2</td>
<td>6.5</td>
<td>—</td>
</tr>
<tr>
<td>Progesterone</td>
<td>3.1</td>
<td>2.1</td>
<td>—</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>1.4</td>
<td>2.1</td>
<td>27.4</td>
</tr>
<tr>
<td>Cortisone</td>
<td>0.9</td>
<td>&lt;0.1</td>
<td>24.4</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1.0</td>
<td>&lt;0.1</td>
<td>—</td>
</tr>
<tr>
<td>Fluoxymesterone</td>
<td>1.6</td>
<td>&lt;0.1</td>
<td>—</td>
</tr>
<tr>
<td>5α,3β-Tetrahydrocortisol</td>
<td>1.3</td>
<td>&lt;0.1</td>
<td>18.2</td>
</tr>
<tr>
<td>11-Epicortisol</td>
<td>0.7</td>
<td>&lt;0.1</td>
<td>14.2</td>
</tr>
</tbody>
</table>

M1 cells were inoculated at 2×10⁵ cells/ml with various steroids. Phagocytosis and enzyme activity were assayed 4 days after incubation of the cells.

### Table II. Effect of Competitive Steroid Interactions on Induction of Lysozyme Activity

<table>
<thead>
<tr>
<th>Steroids (5×10−6M)</th>
<th>Lysozyme activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−Dexamethasone</td>
</tr>
<tr>
<td>None</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>27.6</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>25.6</td>
</tr>
<tr>
<td>11β-Hydroxyprogesterone</td>
<td>7.1</td>
</tr>
<tr>
<td>11-Deoxycortisol</td>
<td>7.9</td>
</tr>
<tr>
<td>Cortisone</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Fluoxymesterone</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>5α,3β-Tetrahydrocortisol</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>11-Epicortisol</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

M1 cells were inoculated at 2×10⁵ cells/ml with each steroid (5×10−6M) and in combination with dexamethasone (5×10−6M). After a 4-day incubation period, lysozyme activities were assayed.
dexamethasone treatment and reached maximum on the 4th day, while the enzyme activity of the cells cultured without dexamethasone remained at the initial level. Then, effect of pH on the induced enzyme activity was examined. The enzyme activity was optimal at pH 3.0 which was similar to that of the non-induced endogenous enzyme.

In order to understand other properties of the enzyme in more detail, the effect of four different kinds of specific protease inhibitors of microbial origin\(^1,22\) on the enzyme was investigated. Although pepstatin, a specific pepsin inhibitor, markedly inhibited the enzyme activity, three other inhibitors scarcely affected it (Table III). This strongly suggests the predominance of cathepsin-D action in this enzyme activity, since cathepsin-D, a pepsin-like acid protease, was reported to be markedly inhibited by pepstatin, while cathepsin-A and -B were refractory.\(^14\) Moreover, no significant difference was found between the \(K_m\) for substrate of the induced enzyme \((2.2 \times 10^{-8}M)\) and that of non-induced enzyme \((2.5 \times 10^{-8}M)\). These results suggest that the enhancement of the enzyme activity is due to an increased activity of the enzyme which is similar to that in the untreated cells and may not be due to the induction of a different type of the enzyme.

### Effect of Dexamethasone on Induction of \(\beta\)-Glucuronidase, Acid Phosphatase, Acid DNase, and Acid RNase Activities in M1 Cells

The time course of induction of \(\beta\)-glucuronidase activity in M1 cells with dexamethasone was examined as shown in Fig. 3. Compared with the kinetics of induction of lysozyme (Fig. 1) and acid protease

**Fig. 2.** Time-course of induction of acid protease activity in M1 cells by dexamethasone

M1 cells were inoculated at a concentration of \(2 \times 10^6\) cells/ml with (●) and without (○) \(5 \times 10^{-6}M\) dexamethasone. Samples were removed at the time indicated and assayed for acid protease activity.

### Table III: Effect of Various Reagents on Acid Protease Activity in M1 Cells

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Final concn. ((\mu g/ml))</th>
<th>Untreated cells</th>
<th>Percent activity(^a) Dexamethasone-treated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leupeptin</td>
<td>1.0</td>
<td>78</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>75</td>
<td>83</td>
</tr>
<tr>
<td>Pepstatin</td>
<td>(2.5 \times 10^{-3})</td>
<td>46</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>(2.5 \times 10^{-2})</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Chymostatin</td>
<td>5</td>
<td>72</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>74</td>
<td>81</td>
</tr>
<tr>
<td>Antipain</td>
<td>2</td>
<td>78</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>79</td>
<td>93</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>40</td>
<td>—</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>—</td>
<td>94</td>
</tr>
</tbody>
</table>

\(^a\) Acid protease activity at pH 3.0 in the absence of any other reagents was taken as 100%.

M1 cells were inoculated at \(2 \times 10^6\) cells/ml, with or without \(1 \times 10^{-6}M\) dexamethasone. After a 4-day incubation period, enzyme solutions were obtained. Acid protease activity was assayed with various reagents.
(Fig. 2) activity, the induction period of the \( \beta \)-glucuronidase activity was longer and the enzyme activity was not detected until 3 days after the treatment with dexamethasone. However, it was significantly increased 4 days later as shown in Fig. 3. On the other hand, the enzyme activity in the untreated

![Graph showing the time-course of induction of \( \beta \)-glucuronidase activity in M1 cells with dexamethasone.](image)

**Fig. 3.** Time-course of induction of \( \beta \)-glucuronidase activity in M1 cells with dexamethasone.

M1 cells were inoculated at \( 2 \times 10^6 \) cells/ml with (●) and without (○) \( 5 \times 10^{-4}M \) dexamethasone. Samples were removed at the time indicated and assayed for \( \beta \)-glucuronidase activity.

**Table IV. Effect of Metabolic Inhibitors on Induction of Phagocytosis and Lysozyme Activity in M1 Cells**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Conc. ((M))</th>
<th>Dexamethasone ((10^{-4}M))</th>
<th>No. of viable cells ((\times 10^6))</th>
<th>Phagocytosis (%)</th>
<th>Lysozyme (\text{units/mg protein})</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>( 10^{-6} )</td>
<td>2.9</td>
<td>2.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>FdU</td>
<td>( 10^{-6} )</td>
<td>–</td>
<td>1.5</td>
<td>60.8</td>
<td>28.5</td>
</tr>
<tr>
<td>BrdU</td>
<td>( 10^{-4} )</td>
<td>+</td>
<td>0.6</td>
<td>3.0</td>
<td>0.1</td>
</tr>
<tr>
<td>( 2 \times 10^{-6} )</td>
<td>+</td>
<td>0.5</td>
<td>72.3</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>Puromycin</td>
<td>( 2 \times 10^{-7} )</td>
<td>+</td>
<td>1.6</td>
<td>28.5</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6</td>
<td>1.4</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>8.0</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>2.6</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.4</td>
<td>48.6</td>
<td>&lt;0.1</td>
<td></td>
</tr>
</tbody>
</table>

M1 cells were inoculated at \( 4 \times 10^5 \) cells/ml in \( 10^{-6}M \) dexamethasone, with or without the inhibitor. Phagocytosis and lysozyme activity were assayed after a 4-day incubation period.

**Effect of Metabolic Inhibitors on Induction of Phagocytosis and Lysozyme Activity in M1 Cells with Dexamethasone**

It was reported that some metabolic inhibitors blocked expression of phagocytosis and locomotive activity induced with a proteinous inducer in ECM. Furthermore, some lysosomal enzyme activities, as well as the phagocytic activity, stimulated by ECM were suppressed by BrdU. We then examined whether or not lysozyme activity induced by glucocorticoid could be inhibited by such metabolic inhibitors (Table IV). Although FdU inhibited growth of the cells, phagocytosis and lysozyme activity were not inhibited, rather they were slightly stimulated. This result suggests that mitosis is not necessarily required for the induction of lysozyme activity, nor for the induction of phagocytosis in M1 cells as reported previously. On the other hand, BrdU inhibited the induction of phagocytosis and lysozyme activity at a concentration that does not interfere with...
growth of the cells. Marked inhibition of the enzyme activity was observed at a higher concentration of BrdU, but the cell growth was significantly inhibited. This result is in agreement with a previous report\(^{20}\) that acid protease and acid phosphatase activity stimulated by ECM was blocked by the same reagent. Puromycin, which is known to inhibit protein synthesis, moderately blocked the induction of phagocytosis and almost completely blocked that of lysozyme activity at a concentration that does not interfere with growth of the cells. In treating M1 cells with these inhibitors alone, the induction of phagocytosis and lysozyme activity was not detected.

**Comparison of Glucocorticoid with ECM in Induction of Lysosomal Enzyme Activity during Differentiation of M1 Cells** Nagata et al.\(^{20}\) reported that in treating M1 cells with ECM, the activities of lysosomal enzymes such as acid phosphatase and acid protease were induced but not those of \(\beta\)-glucuronidase and acid DNase. The effect of dexamethasone and ECM on the induction of activity of some lysosomal enzymes was examined under the same conditions (Table V). Both dexamethasone and ECM induced lysozyme, acid protease, and \(\beta\)-glucuronidase activity during differentiation of M1 cells. Although enhancement of \(\beta\)-glucuronidase activity by ECM was slight, it was always observed in a series of experiments (18\(^{\sim}\)21\% increase, five separate experiments). However, no stimulation of acid phosphatase activity was observed even 5 days after treating the cells with dexamethasone and ECM. Thus, no significant difference between dexamethasone and ECM in the induction of lysosomal enzyme activity was observed in the clones we tested.

**Effect of Dexamethasone on Induction of Phagocytosis and Lysosomal Enzyme in Dexamethasone-resistant M1 Cells** DR-6, a dexamethasone-resistant clone which could not differentiate even in a high concentration of dexamethasone, was isolated from dexamethasone-sensitive M1 cells. The effect of dexamethasone on induction of lysosomal enzyme activity in the resistant cells was examined (Table V). The induction of phagocytic and lysozyme activity in the cells was not detected in the presence of \(2 \times 10^{-6} M\) dexamethasone. The activities of \(\beta\)-glucuronidase and acid phosphatase in DR-6 cells treated with dexamethasone remained in a slightly lower level than those of the untreated DR-6 cells. Acid protease in the

| Table V. Effect of Dexamethasone and ECM on Phagocytosis and Lysosomal Enzyme Activity |
|----------------------------------------|--------|--------|--------|--------|--------|
| Cell clone   | Treatment | Phagocytosis (%) | Lysozyme (units/mg protein) | Acid protease (μg hemoglobin/min/ mg protein) | \(\beta\)-Glucuronidase (nmol \(p\)-nitrophenol/min/mg protein) | Acid phosphatase (nmol \(p\)-nitrophenol/min/mg protein) |
| DS-4        | —        | 4.9     | <0.1   | 23.0   | 4.7    | 14.3   |
| Dexamethasone (10\(^{-4}\)M) | —        | 56.4    | 40.0   | 41.0   | 9.4    | 14.0   |
| ECM (50%)   | —        | 64.3    | 22.9   | 33.2   | 5.6    | 14.3   |
| DR-6        | —        | 0       | <0.1   | 20.8   | 7.2    | 8.6    |
| Dexamethasone (2 \times 10\(^{-5}\)M) | —        | 0       | <0.1   | 22.5   | 5.0    | 6.8    |
| Peritoneal macrophages | —        | 119.1   | 108.7  | 26.7   | 50.1   |

M1 (DS-4) cells were inoculated at \(2 \times 10^{6}\) cells/ml with \(10^{-4} M\) dexamethasone or 50\% (v/v) ECM. DR-6 cells were inoculated at \(2 \times 10^{6}\) cells/ml, with or without \(2 \times 10^{-5} M\) dexamethasone. Phagocytosis and enzyme activity were assayed 4 days after incubation of the cells. Mouse peritoneal macrophages were obtained from SL strain mice.
cells was not significantly stimulated by steroids.

Under the same experimental conditions, phagocytosis and lysosomal enzyme activity in the dexamethasone-sensitive DS-4 cells were markedly induced by dexamethasone or ECM, although the lysosomal enzyme activities were less than those in normal peritoneal macrophages (Table V).

**DISCUSSION**

M1 cells were induced by glucocorticoids to differentiate into cells which appeared to be similar to macrophages and granulocytes from normal hematopoietic organs. Glucocorticoids are assumed to influence responsive cells by binding to a cytoplasmic receptor and subsequent interaction of the hormone-receptor complex with some component of nuclear chromatin. We recently detected glucocorticoid receptors in M1 cells and determined binding affinities of various steroids for the cytoplasmic receptors in M1 cells which were closely related to the activities of these compounds to induce differentiation of M1 cells. These results suggest that the binding reaction of glucocorticoids to the cytoplasmic receptor is necessary for normal differentiation of M1 cells with glucocorticoids.

In the present experiments, lysozyme activity was induced and increased progressively with higher dexamethasone concentrations (Fig. 1). Similar dose–response curve was obtained in the induction of phagocytic activity with dexamethasone, although the induction of phagocytic activity was faster than that of the lysozyme activity. Therefore, the induction of lysozyme activity and phagocytosis in M1 cells with glucocorticoids was found to be closely related. Moreover, the effect of competitive interaction among different steroids on the induction of lysozyme activity suggests that specific steroid hormone receptors are involved in the induction of the enzyme activity in M1 cells.

In preparation of the organelles from M1 cells by differential centrifugation of the cell homogenate, at least 50% of the total lysozyme content in the cells was found in the fraction that sedimented at 11,000g for 20 min. This indicates that the enzyme was mainly distributed in the lysosomal fraction of the cells. It was previously reported that activation of the lysosomal enzymes in M1 cells with ECM was associated with phagocytosis and it was independent of mere injury to the cells. Such injurious activation of the enzyme was also not found in the induction of lysozyme activity with glucocorticoids.

Although lysozyme activity in the culture medium of M1 cells could not be detected even 5 days after culture of the cells, the activity was definitely found in the culture medium of the cells treated with dexamethasone. Therefore, total activity of lysozyme induced from cells with dexamethasone was significantly higher than that in intracellular enzyme alone. On the other hand, activity of the enzyme in the culture medium of the dexamethasone-resistant cells (DR-6) with $2 \times 10^{-5} M$ dexamethasone could not be detected.

The results of the present experiments also indicate that general properties of acid protease from the dexamethasone-treated and untreated cells belong to cathepsin D-like protease which is sensitive to pepstatin and is active at acidic pH.

Nagata *et al.* reported that acid protease and acid phosphatase activity in M1 cells was induced with ECM but not that of β-glucuronidase. On the other hand, dexamethasone induced lysozyme, acid protease, and β-glucuronidase activity in the cells but not that of acid phosphatase. The difference in the activity of enzymes induced in our cells and those in the cells in previous experiments may be partly due to a clonal difference of M1 cells.

The lysozyme activity could not be detected in untreated M1 cells. However, the enzyme activity in M1 cells was clearly manifested
LYSOSOMAL ENZYMES AND DIFFERENTIATION

in the course of differentiation of the cells with glucocorticoids. Therefore, lysozyme activity is a fairly suitable biochemical marker for pursuing the differentiation of M1 cells in this experimental system and will be used to study further molecular mechanisms of differentiation in M1 cells.

This work was partly supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, and from the Ministry of Health and Welfare.

(Received May 2, 1977)

REFERENCES