Role of ADP-Ribosyl Transferase in Differentiation of Human Granulocyte-Macrophage Progenitors to the Macrophage Lineage

By G. E. Francis, D. A. Gray, J. J. Berney, M. A. Wing, J. E. T. Guimaraes, and A. V. Hoffbrand

ADENOSINE DIPHOSPHATE-ribosyl (ADP-ribosyl) transferase is a chromatin-bound enzyme catalyzing the transfer of ADP-ribose from NAD⁺ to chromatin proteins. The physiologic function of this covalent modification of chromatin has not been fully established, but roles in both DNA repair and in differentiation have been proposed. We demonstrate that three specific inhibitors of ADP-ribosyl transferase (5-methylnicotinamide, 3-methoxybenzamide, 3-aminobenzamide) inhibit differentiation of human granulocyte-macrophage progenitor cells to the macrophage lineage. Differentiation to the neutrophil-granulocyte lineage is much less affected. The inhibition of macrophage differentiation seems to relate to the ability of these compounds to inhibit ADP-ribosyl transferase. A structural analogue (3-methoxybenzoic acid), which is not inhibitory for the enzyme, did not inhibit macrophage differentiation. Additional evidence for a role of ADP-ribosyl transferase in the differentiation of granulocyte-macrophage progenitors was obtained from experiments in which enzyme activity levels were measured in permeabilized marrow cells. Marrow cell ADP-ribosyl transferase activity increased after 3-hr stimulation by the differentiation/proliferation stimulus—granulocyte-macrophage colony-stimulating activity (GM-CSA). Unstimulated marrow cells showed low or undetectable levels of enzyme activity.

The cloning of bone marrow cells in semisolid agar cultures provides an opportunity to observe the committed progenitor cell for granulocytes and macrophages (CFU-GM) proliferating and differentiating in vitro. Cytochemical staining for nonspecific and chloroacetate esterase activity, performed on whole culture gels, permits accurate typing of clone cells and rapid classification of large numbers of stained clones. We used this system and three different, highly specific, inhibitors of ADP-ribosyl transferase (5-methylnicotinamide, 3-aminobenzamide, 3-methoxybenzamide) to determine whether differentiation in these two closely related hemopoietic lineages, the neutrophil-granulocyte and monocyte-macrophage, involves ADP-ribosylation.

The results show that differentiation down the mon-
cyte-macrophage pathway, to the stage where cells acquire nonspecific esterase (NSE) activity, is readily blocked by all three ADP-ribosyl transferase inhibitors tested, whereas granulocytic differentiation is much less sensitive to such inhibitors. Furthermore, stimulation of granulocyte-macrophage progenitors by the proliferation/differentiation stimulus, granulocyte-macrophage colony-stimulating activity (GM-CSA), produced a rapid increase in ADP-ribosyl transferase activity.

MATERIALS AND METHODS

Bone Marrow Cultures

The double-layer semisolid agar technique of Pike and Robinson was used throughout. Bone marrow cells were obtained from healthy human volunteers (with the approval of the Ethical Practices Committee of the Royal Free Hospital). Samples were depleted of neutrophils by centrifugation at 400 g for 40 min over Lymphocyte Separation Medium (Flow Labs Ltd., Irvine, UK) and of CSA-producing cells by adherence and were cultured at 0.7-2.0 × 10^5 cells/ml. The ADP-ribosyl transferase inhibitors used were: 3-aminobenzamide (Sigma Ltd, Poole, UK), 3-methoxybenzamide (Aldrich Chemical Co.), and 5-methylisoxazole (gift of Drs. A. Johnstone and G. Williams). These were diluted in the standard tissue culture medium used for the cultures (McCoy's 5A supplemented with 15% fetal calf serum and antibiotics, Flow Labs Ltd.).

Stock solutions (10 mM) were made up usually on the day of use, and stored at -20°C. Where required, inhibitor stock was adjusted to that of the other stock solutions and the diluent control was used. The double-layer semisolid agar technique of Pike and Robinson was used for scoring. We have demonstrated that this “best of three approach” is a more reliable means of providing clone formation at plateau levels than the use of feeder layers from a single donor.33

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medium was made from the same batch of tissue culture medium and incubated for the same period, but without leukocytes (unconditioned medium). The pH of the conditioned medium was adjusted to that of the unconditioned medium using approximately 2 ml sodium bicarbonate solution [Flow Ltd., 7.5% (w/v)] per 100 ml of medium.

RESULTS

Figure 1 summarizes the results of day 7-8 marrow cultures in which bone marrow cells from four different healthy donors were cultured in the presence of three different inhibitors of ADP-ribosyl transferase (5-methyl nicotinamide, 3-methoxybenzamide, and 3-amino benzamide). The inhibitors showed different effects on the three types of clone, granulocyte-macrophage (GM), pure macrophage (M), and pure neutrophil-granulocyte (G). The results of each type of clone are expressed as a percent of the untreated control value for the same type of clone (these values are given in Table 1). With each of the three inhibitors, formation of clones containing macrophages (GM and M clones) was profoundly inhibited, whereas G-clone formation was not.

Each of the 4 marrows of Fig. 1 was also tested at an additional culture time point (separate cultures), either day 5 (marrows 1 and 2) or day 14 (marrows 3 and 4). The results are given in Table 1. Neutrophil-granulocytes and macrophages have different kinetics of production in vitro, the latter somewhat slower than the former, so that a proportion of apparently pure G-clones in day-5 cultures later develop a macrophage day-7 GM-clones, neutrophils have degenerated by day 14 so that they appear as pure M-clones.

With 2.5 mM 5-methyl nicotinamide and 2.5 mM 3-methoxybenzamide, there was profound inhibition of formation of macrophages at all culture time points. The difference in effect of inhibitors on day-5 and day-8 G-clones (marrows 1 and 2) may reflect the difference in the nature of these two clone categories. Between days 5 and 8 of culture, 2.5 mM 3-amino benzamide produced a similar effect to the other inhibitors, with profound inhibition of macrophage but minimal or no inhibition of granulocyte production. In contrast, in the two marrow cultures that were continued for 14 days, the inhibitory effect of 3-amino benzamide was partially reversed. This may reflect instability of this compound on prolonged incubation at 37°C or consumption of inhibitor during the course of the experiment.

The number of pure granulocyte clones actually increased above the 100% control values in 8/12 experiments of Table 1. This effect was observed with all three inhibitors. Comparison of total clone numbers and proportions of GM- and G-clones (as in the example in Fig. 2) suggested that this increase in G-clones reflected failure of macrophage formation by bipotent cells, which had formed granulocyte-macrophage clones in the control cultures (since the total clone number was not increased and the granulocyte clone total in the treated cultures was never significantly greater than the granulocyte-macrophage plus granulocyte clone total in control cultures).

The extent to which the three agents were inhibitory for total clone formation varied from marrow to marrow, and this was largely due to variation in the response of G-clones. This occurred despite the fact that all marrows were obtained from healthy donors and was not confined to any particular inhibitor. Marrow three, for example, was particularly sensitive to inhibition by 3-methoxybenzamide, whereas other marrows were more sensitive to 5-methyl nicotinamide. Even where neutrophil-granulocyte clone formation was suppressed, the percent inhibition of macrophage-containing clones (pure macrophage and mixed granulocyte-macrophage) exceeded the percent inhibition of pure neutrophil-granulocyte clones at all drug doses, due to a relative sparing of granulocyte clones (Fig. 3).

This inhibitory effect of macrophage production appears to be related to the ability of these compounds to inhibit ADP-ribosyl transferase. The structural analogue, 3-methoxy benzoic acid, which is not inhibitory to the enzyme, failed to inhibit macrophage production (Fig. 4).

These results suggested that ADP-ribosylation of chromatin proteins, catalyzed by ADP-ribosyl transferase, might play an important role in the differentia-
Table 1. Effect of ADP-Ribosyl Transferase Inhibitors on Granulocyte-Macrophage Clone Formation

<table>
<thead>
<tr>
<th>Clones/10^4 Nucleated Cells (% Total)</th>
<th>Bone Marrow 1</th>
<th>Bone Marrow 2</th>
<th>Bone Marrow 3</th>
<th>Bone Marrow 4</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Day 5</td>
<td>Day 8</td>
<td>Day 5</td>
<td>Day 8</td>
</tr>
<tr>
<td>Untreated control</td>
<td>1.062 ± 60</td>
<td>899 ± 45</td>
<td>488 ± 40</td>
<td>388 ± 61</td>
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<tr>
<td>G-clones*</td>
<td>982.6</td>
<td>413.5</td>
<td>472.1</td>
<td>356.2</td>
</tr>
<tr>
<td>(92.5%)</td>
<td>(46.0%)</td>
<td>(96.7%)</td>
<td>(91.8%)</td>
<td>(91.6%)</td>
</tr>
<tr>
<td>M-clones*</td>
<td>78.1</td>
<td>116.9</td>
<td>15.4</td>
<td>18.2</td>
</tr>
<tr>
<td>(7.4%)</td>
<td>(13.0%)</td>
<td>(3.2%)</td>
<td>(4.7%)</td>
<td>(4.7%)</td>
</tr>
<tr>
<td>GM-clones*</td>
<td>1.3</td>
<td>368.6</td>
<td>0.5</td>
<td>13.6</td>
</tr>
<tr>
<td>(0.1%)</td>
<td>(41.0%)</td>
<td>(0.1%)</td>
<td>(3.5%)</td>
<td>(6.5%)</td>
</tr>
</tbody>
</table>

Clones/10^4 Nucleated Cells (% Untreated Control)

2.5mM 5-methyl-nicotinamide

<table>
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<tr>
<th>Total clones</th>
<th>404.3</th>
<th>565.5</th>
<th>80.2</th>
<th>369.0</th>
<th>465.9</th>
<th>360.2</th>
<th>1.780.1</th>
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<tr>
<td>G-clones</td>
<td>403.8</td>
<td>559.9</td>
<td>78.7</td>
<td>367.4</td>
<td>465.9</td>
<td>320.6</td>
<td>1.744.2</td>
<td>192.6</td>
</tr>
<tr>
<td>(41.1%)</td>
<td>(35.4%)</td>
<td>(16.7%)</td>
<td>(103.1%)</td>
<td>(93.6%)</td>
<td>(133.6%)</td>
<td>(92.0%)</td>
<td>(90.6%)</td>
<td>(7.4%)</td>
</tr>
<tr>
<td>M-clones</td>
<td>0.5</td>
<td>5.6</td>
<td>1.5</td>
<td>1.3</td>
<td>0</td>
<td>26.8</td>
<td>9.2</td>
<td>7.8</td>
</tr>
<tr>
<td>(0.6%)</td>
<td>(4.8%)</td>
<td>(9.6%)</td>
<td>(7.2%)</td>
<td>(8.7%)</td>
<td>(8.7%)</td>
<td>(9.3%)</td>
<td>(1.8%)</td>
<td>(0.6%)</td>
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<tr>
<td>GM-clones</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12.8</td>
<td>26.7</td>
<td>2.0</td>
</tr>
<tr>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
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2.5mM 3-methoxy-benzamide

<table>
<thead>
<tr>
<th>Total clones</th>
<th>621.2</th>
<th>660.8</th>
<th>300.8</th>
<th>347.9</th>
<th>36.7</th>
<th>28.6</th>
<th>446.8</th>
<th>314.4</th>
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<tr>
<td>G-clones</td>
<td>618.2</td>
<td>611.5</td>
<td>297.8</td>
<td>343.2</td>
<td>35.5</td>
<td>17.8</td>
<td>444.4</td>
<td>217.1</td>
</tr>
<tr>
<td>(58.5%)</td>
<td>(73.5%)</td>
<td>(61.6%)</td>
<td>(89.7%)</td>
<td>(96.2%)</td>
<td>(3.9%)</td>
<td>(7.4%)</td>
<td>(25.9%)</td>
<td>(102.0%)</td>
</tr>
<tr>
<td>M-clones</td>
<td>3.0</td>
<td>43.0</td>
<td>3.0</td>
<td>3.7</td>
<td>1.2</td>
<td>9.9</td>
<td>2.4</td>
<td>7.9</td>
</tr>
<tr>
<td>(3.8%)</td>
<td>(36.8%)</td>
<td>(19.6%)</td>
<td>(20.6%)</td>
<td>(8.0%)</td>
<td>(29.5%)</td>
<td>(24.5%)</td>
<td>(18.3%)</td>
<td>(0.6%)</td>
</tr>
<tr>
<td>GM-clones</td>
<td>0</td>
<td>6.3</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>17.6</td>
<td>(0%)</td>
</tr>
<tr>
<td>(0%)</td>
<td>(1.8%)</td>
<td>(0%)</td>
<td>(7.2%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(37.0%)</td>
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2.5mM 3-amino-benzamide

<table>
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<tr>
<th>Total clones</th>
<th>579.4</th>
<th>894.3</th>
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<th>390.3</th>
<th>766.0</th>
<th>535.7</th>
<th>1.563.0</th>
<th>430.8</th>
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<td>G-clones</td>
<td>570.4</td>
<td>814.1</td>
<td>381.0</td>
<td>385.8</td>
<td>762.1</td>
<td>199.8</td>
<td>1.547.0</td>
<td>159.5</td>
</tr>
<tr>
<td>(58.1%)</td>
<td>(96.9%)</td>
<td>(80.7%)</td>
<td>(108.2%)</td>
<td>(84.8%)</td>
<td>(83.3%)</td>
<td>(90.1%)</td>
<td>(75.0%)</td>
<td>(78.3%)</td>
</tr>
<tr>
<td>M-clones</td>
<td>9.0</td>
<td>71.4</td>
<td>3.5</td>
<td>2.8</td>
<td>0</td>
<td>230.8</td>
<td>16.0</td>
<td>221.8</td>
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<tr>
<td>(11.5%)</td>
<td>(99.5%)</td>
<td>(78.8%)</td>
<td>(100.6%)</td>
<td>(56.1%)</td>
<td>(71.4%)</td>
<td>(79.2%)</td>
<td>(61.8%)</td>
<td>(16.2%)</td>
</tr>
<tr>
<td>GM-clones</td>
<td>0</td>
<td>8.8</td>
<td>0</td>
<td>1.8</td>
<td>3.9</td>
<td>105.1</td>
<td>0</td>
<td>49.5</td>
</tr>
<tr>
<td>(0%)</td>
<td>(2.4%)</td>
<td>(0%)</td>
<td>(13.1%)</td>
<td>(4.2%)</td>
<td>(51.9%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(101.4%)</td>
</tr>
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</table>

*Proportions of G-, M-, and GM-clones were estimated from differential counts on dual esterase-stained gels (from 200 to 4 clones on each of 2-4 replicate gels).
†Means ± SEM of clone counts on 2-4 culture gels.

Fig. 2. Inhibition of GM and M-clones was accompanied in this and other experiments (see Table 1), by an absolute increase in the number of G-clones. The total number of clones was obtained from counts on triplicate day-8 culture gels and the proportions of GM-, M-, and G-clones was calculated from differential counts on 200 consecutive clones.
tion of the progenitor cells down the macrophage pathway. We therefore tested the effect of the proliferation/differentiation stimulus GM-CSA on the ADP-ribosyl transferase activity of bone marrow cells. Neutrophil-depleted, CSA-producing (adherent) cell-depleted bone marrow cells were exposed to leukocyte conditioned medium (80%, v/v) as a source of GM-CSA or to unconditioned medium for unstimulated cell controls. A 3-hr incubation period was chosen since, at longer time points, cells start to die in the absence of

Fig. 3. Representative dose-response curves. The upper two panels show results of day-7 cultures for the same marrow, and the lower left and right day-8 and day-7 cultures, respectively, of two different marrow samples. Results are means ± SEM of clone counts on 2-4 replicate culture gels and are expressed as a percentage of the number of that type of clone formed in the absence of the inhibitor (1,005 ± 195 G-clones, 276 ± 154 M-clones, and 96 ± 37 GM-clones for the 3 marrow).

Fig. 4. Comparison of the effects of benzamide inhibitors of ADP-ribosyl transferase and a structural analogue, 3-methoxybenzoic acid, not inhibitory for this enzyme, on differentiation of progenitor cells to the neutrophil-granulocyte and macrophage lineages. Cultures were incubated for 8 days, and 2 mM concentration was used for all three agents. Only the compounds that inhibit the enzyme (3-aminobenzamide and 3-methoxybenzamide) had an inhibitory effect on differentiation. (A) untreated, (B) 3-amino- benzamide, (C) 3-methoxybenzamide, (D) 3-methoxybenzoic acid.

Fig. 5. The effect of GM-CSA and inhibitor on ADP-ribosyl transferase activity levels. Light density (-1.077 g · cm⁻³) nonadherent bone marrow cells were assayed after 3 hr incubation in 80% (v/v) conditioned medium containing GM-CSA (stimulated cells) or after incubation in medium alone (unstimulated cells). The incorporation of radioactivity from "H-NAD" into acid-insoluble material (ADP-ribose) was measured in the presence (+) and absence (−) of inhibitor (5-methylnicotinamide). The dotted line shows the level of nonspecific incorporation indicated by the results on quadruplicate samples of unstimulated cells to which trichloroacetic acid was added at time 0. Representative example of two independent experiments.
of these compounds to inhibit ADP-ribosyl transferase activity of unstimulated and stimulated cells in the presence and absence of inhibitor (5-methylnicotinamide, 2.5 mM). The time zero control indicates the nonspecific label incorporation. Unstimulated cells had activities not significantly above background. After 3-hr exposure to GM-CSA, there was an increase in enzyme activity. The stimulated cells showed a 3.4-fold increase in the incorporation of ADP-ribose from NAD$^+$ above the value obtained for unstimulated cells (an over tenfold increase if background counts are subtracted). The ability of 5-methylnicotinamide to inhibit this increase in activity suggests that this increased incorporation of label from NAD$^+$ was due to increased ADP-ribosyl transferase activity. A second assay performed with different marrow cells, but with the same conditioned medium (data not shown), showed similar results, with unstimulated cells having activities not significantly above background and stimulated cells showing a 4.3-fold increase in NAD$^+$ incorporation above that of unstimulated cells. Again, the increase in activity in this second experiment was inhibited by 5-methylnicotinamide.

DISCUSSION

The results showed that three inhibitors of the enzyme ADP-ribosyl transferase (5-methylnicotinamide, 3-methoxybenzamide, 3-aminobenzamide) inhibited differentiation of granulocyte-macrophage progenitor cells to the macrophage lineage. The three agents used are highly specific inhibitors of ADP-ribosyl transferase, and the concordant results obtained here with different types of inhibitor reduce the likelihood of other inhibitory actions of these agents being responsible for the observed effect on differentiation. For example inhibitory activity for NAD glycohydrolase is shown by 5-methylnicotinamide but not by 3-aminobenzamide and cannot, therefore, be responsible for the inhibitory effect on differentiation.

Two additional types of experiment suggested that this inhibition of differentiation was due to the ability of these compounds to inhibit ADP-ribosyl transferase in marrow progenitor cells and was not due to a nonspecific action of the agents or to indirect effects. First, structural analogues, such as nicotinic acid and benzoic acid derivatives, which are not inhibitory for ADP-RT, provide a check for specificity of action. The structural analogue 3-methoxybenzoic acid did not inhibit macrophage differentiation. Secondly, marrow cell ADP-ribosyl transferase assays suggested that increased enzyme activity precedes differentiation. It was shown, using permeabilized marrow cells, that an increase in ADP-ribosyl transferase activity occurs within hours of exposure of cells to the specific differentiation/proliferation stimulus GM-CSA. That this increase in uptake of label from NAD$^+$ was due to an increase in enzyme activity was suggested by the observation that it was abolished if the stimulated cells were exposed to specific inhibitor (5-methylnicotinamide). The time scale of this increase in enzyme activity indicates that differentiation is preceded by an increase in ADP-ribosyl transferase, since the appearance of macrophage-containing clones only occurs after 3–4 days of culture in the presence of GM-CSA.

The observation that marrow cells, depleted of CSA-producing cells and exposed to a cell-free source of GM-CSA, not only have increased ADP-RT activity but also that this is susceptible to inhibition by 5-methyl nicotinamide, suggests that the effects observed here on differentiation were due to a direct effect on progenitor cells and were not mediated indirectly via feeder layer CSA-producing cells.

There is an increasing body of evidence that ADP-ribosylation of chromatin proteins is involved in eukaryote differentiation. Since ADP-ribosyl transferase has a role in regulation of DNA ligation, and DNA strand breaks occur during differentiation, it has been suggested that the role in differentiation is in strand rejoining, necessary when specific gene expression requires gene rearrangement or DNA transposition. The wide variety of cell lineages and species studied in these reports suggests ubiquitous involvement of ADP-ribosylation in differentiation. This report is, as far as we are aware, the first in which differentiation by a progenitor cell with more than one lineage option has been studied in clonal assays, revealing an effect of ADP-RT inhibitors on the direction of differentiation. Such an effect has been suggested on indirect evidence from the effect of ambient NAD levels on the muscle and cartilage differentiation of embryonic chick limb mesenchymal cells, although direct evidence of change in the direction of differentiation of individual bipotent progenitor cells was not obtained. This raises the possibility that control of ADP-ribosylation may, in addition to the generalized role in differentiation already proposed, have a role in the control of which lineage options of a multipotent progenitor are expressed.

The committed granulocyte-macrophage progenitor cell grown in the semisolid agar system gives rise to clones of granulocytes and macrophages. Marrow samples also contain cells with restricted potential giving rise to only one lineage. However, the kinetics of production of the two cell types in culture and the stochastic nature of cell division/differentiation within
individual clones means that a proportion of single lineage clones will have arisen from bipotent progenitors. There is, however, evidence from separation studies to suggest that the majority of single lineage clones at day 7 of culture arise from cell populations functionally distinct from that producing mixed granulocyte-macrophage clones, presumably committed unipotent progenitor cells. The observation made here of an increase in the number of pure granulocyte clones above control values in some experiments suggests that the macrophage component of some granulocyte-macrophage clones had failed to form in the presence of the inhibitors. The observation that the number of pure macrophage clones declined usually to a lesser extent than granulocyte-macrophage clones suggests that it may be a differentiation event very close to the bifurcation of the two pathways that involves ADP-ribosylation and that some macrophage lineage cells, once formed, do not have their further proliferation/differentiation as strongly inhibited by the ADP-ribosyl transferase inhibitors.

Experiments using murine GM colony-forming cells have shown evidence of irreversible commitment to the macrophage lineage after 2 or 3 cell divisions have taken place in the presence of M-CSA. Such an irreversible change in a cell’s potential could be brought about by gene rearrangement. There is some precedent for this in recent data, which suggest that there are some differences in the arrangement of genetic material between the different tissues of an individual. The results of this study suggest that ADP-ribosyl-transferase-mediated regulation of DNA ligation might be involved at specific commitment sites in the differentiation pathways of multipotent progenitor cells to different lineages. However, although previous reports suggest ubiquitous involvement of ADP-ribosylation in eukaryotic differentiation, this report shows that the enzyme is not necessarily involved to the same extent in the differentiation of even closely related alternative lineages for the same progenitor cell.

ADP-ribosyl transferase activity has recently been demonstrated in membranes, and it has been suggested that this enzyme has a role via adenylylate cyclase in hormonal regulation by polypeptide hormones. Although it is possible that the inhibitors used in this study produced their effects by inhibiting the membrane enzyme, the parallels between our report and those of workers studying differentiation in other mammalian cells indicate that it is more likely to be inhibition of nuclear ADP-ribosyl transferase that is responsible for inhibition of macrophage differentiation.

The observation in other systems that it is primarily differentiation rather than proliferation that is inhibited is in agreement with the observation, made here, of profound inhibition of macrophage clone formation in the presence of much more modest degrees of inhibition of both clone number (Fig. 1 and Table 1) or of clone size (data not shown). There are, however, reports of effects of ADP-ribosyl transferase inhibitors on proliferation, and we cannot exclude the possibility that ADP-ribosylation might specifically regulate the proliferative activity of cells engaged in terminal differentiation to the monococyte-macrophage lineage.

Whether the cells that are prevented from differentiation to the macrophage lineage die, remain quiescent, or produce neutrophils is not revealed by our data, since all three alternatives could produce reduction of macrophage-containing clones and “conversion” of some GM-clones to G-clones. Whichever of these three possibilities is correct, it will be interesting to explore the possible use of ADP-RT inhibitors in leukemias with monocyte differentiation (acute myeloid leukemia FAB groups M4 and M5 and chronic myelomonocytic leukemia). Since differentiation to the monococyte-macrophage lineage does not, unlike that to the granulocyte lineage, result in loss of proliferative capacity, ADP-RT inhibitors might, by inhibiting monocyte differentiation, produce a considerable reduction in proliferating leukemic cell mass. Certainly, since the site of action of ADP-RT inhibitors is before the stage of NSE expression, and since the bulk of the leukemic cells in these cases are NSE+, these agents should affect their leukemic clones. Preliminary investigations of the effect of ADP-RT inhibitors on leukemic cell differentiation and proliferation in vitro is now in progress.

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