Induction of Human Granulocyte Differentiation
In Vitro by Ubiquitin and Thymopoietin


Human bone marrow cells were separated according to density by centrifugation on Ficoll-Hypaque gradients and then according to size by velocity sedimentation. This procedure resulted in fractions enriched for immature granulocytes, mature granulocytes, and lymphocytes. Cells in these fractions were analyzed for their expression of certain surface and functional differentiation markers and for their ability to respond to thymopoietin and ubiquitin with the expression of additional differentiation markers. A higher percentage of band form and segmented granulocytes than of more immature granulocytes expressed complement receptors on their surfaces. Thymopoietin and ubiquitin induced a significant percentage of the cells in the immature granulocyte fraction to express this marker. These data suggested that the complement receptor may be viewed as a differentiation marker on human granulocytes, the expression of which can be induced in vitro by thymopoietin and ubiquitin. Furthermore, fractions containing predominantly band form granulocytes were induced by ubiquitin (but not thymopoietin) to develop the capacity to respond to chemotactic agents, and cell fractions containing predominantly myelocytes and metamyelocytes were induced by thymopoietin and ubiquitin to develop the capacity to phagocytose latex particles. These findings indicated that thymopoietin and ubiquitin, two agents known to induce a number of stages of human and mouse lymphocyte differentiation, are also capable of inducing some stages of human granulocyte differentiation in vitro.

DEMONSTRATION that a functioning thymus is necessary for the normal development of cellular immunity has led to studies on the sequential steps of lymphocyte differentiation and their regulation. That thymic hormones are involved in some aspects of this process has been suggested by the ability of implants of thymus in a cell-impermeable chamber to restore immunocompetence to neonatally thymectomized animals. A number of factors have been isolated from mammalian thymuses which are capable of influencing various aspects of murine lymphocyte differentiation in vitro and in vivo.

Goldstein has described the isolation of thymopoietin, a 5562-dalton polypeptide of defined amino acid sequence which is secreted by thymic epithelial cells. Thymopoietin induces in vitro the expression of certain differentiation antigens on immature mouse T-lymphocyte precursors. In addition to inducing thymocyte cell-surface antigens, thymopoietin at higher concentrations also...
effects B-cell differentiation.\textsuperscript{9} Ubiquitin is an 8451-dalton polypeptide, isolated by Goldstein, whose amino acid sequence is also known, and it has a widespread tissue distribution.\textsuperscript{6} Ubiquitin induces both T- and B-cell differentiation markers on precursors of mouse lymphoid cells.

A problem common to most differentiation studies in vitro has been the heterogeneity of the cell populations assayed. To identify the cells being induced by these agents, we have linked two cell-separation systems giving more homogeneous cell fractions than those obtained by discontinuous density gradients. In the course of our studies on the induction of surface markers on human bone marrow lymphocytes by thymopoietin and ubiquitin, we have observed striking effects of these agents on granulocytes.\textsuperscript{10} We report here that the complement receptor can be used as a differentiation marker for granulocytes and that the expression of this marker on immature granulocytes can be induced by thymopoietin and ubiquitin. Ubiquitin is also capable of inducing the functional differentiation of granulocytes since short-term incubation of immature marrow granulocytes with a low concentration of ubiquitin confers on these cells the capacity to respond to a chemotactic agent and to phagocytose latex particles, functions which they are not able to perform prior to exposure to these agents.

\textbf{MATERIALS AND METHODS}

\textit{Materials}

Medium RPMI-1640 containing 50 U/ml penicillin and 50 μg/ml streptomycin, (RPMI-PS), minimum essential medium-Joklik modified (MEM-J), and heat-inactivated gamma globulin-free fetal calf serum (FCS) were purchased from the Grand Island Biological Company, Grand Island, N.Y. Ficoll was purchased from Pharmacia, Uppsala, Sweden, Hypaque-M from Winthrop Labs, New York, N.Y., heparin from Upjohn, Kalamazoo, Mich., and the Staput sedimentation apparatus from Johns Scientific, Toronto, Canada. Latex particles (uniform 0.8 μm diameter) were purchased from Dow Chemical, Indianapolis, Ind., sheep red blood cells (SRBC) from Flow Labs, Rockville, Md., and purified 19S rabbit anti-SRBC from BBL, Cockeysville, Md.

\textit{Methods}

\textit{Bone marrow cell separation.} Bone marrow was obtained, with informed consent, from seven healthy volunteers (21-28 yr old) by multiple 0.5-ml aspirations from the posterior iliac crest using heparin at a final concentration of 20 U/ml. Five ml of bone marrow were then diluted with 10 ml of RPMI-PS and this suspension was layered over Ficoll-Hypaque (F-H), prepared according to the technique of Boyum.\textsuperscript{11} The tubes were centrifuged at 450 g for 30 min at 20°C. Plasma was removed first and then cells were recovered from both the F-H/plasma interface and the layer of F-H above the erythrocyte pellet. Cells were washed three times by centrifugation at 4°C with RPMI-PS and then resuspended to a concentration of 1 x 10^7 cells/ml in MEM-J containing 0.1 mg/ml of Ficoll. Cell viability was 98%-100% as determined by trypan blue exclusion.

The marrow cells were then separated by velocity sedimentation at unit gravity at 4°C according to the method of Miller and Phillips.\textsuperscript{12} First, 50 ml of MEM-J were loaded into an 11-cm sedimentation chamber. Next, 15 ml of MEM-J + 0.1 mg/ml of Ficoll containing bone marrow cells at 1 x 10^7/ml were added. Finally, a 525-ml buffered step gradient was introduced under the cells using MEM-J containing Ficoll at a concentration of 0.2 mg/ml in the first (2 cm) gradient chamber, 0.5 mg/ml in the second (11 cm) chamber, and 1.0 mg/ml in the third (11 cm) chamber. The entire loading procedure took 30 min. Cells were allowed to sediment at 4°C for 4.5 hr and were then collected in 15-ml fractions at a flow rate of 30 ml/min. The cell concentration in each fraction was determined in a hemocytometer and adjacent fractions were pooled when necessary. Cells were then washed twice in RPMI-PS.
Peripheral blood cell separation. Mononuclear cells were isolated from peripheral blood by F-H centrifugation as described previously. Peripheral blood granulocytes were obtained by resuspending the pellet obtained after F-H centrifugation in 2 volumes of RPMI-PS and 1 volume of 6% (w/v) dextran in 0.9% (w/v) saline. This mixture was allowed to sediment at 1 g for 30 min at 37°C. Granulocytes were harvested from the slowly sedimenting layer, washed twice in RPMI-PS, and resuspended to 3 x 10^6 cells/ml.

Cell incubation conditions. Aliquots of 1.5 x 10^6 cells from each fraction in 1 ml of RPMI-PS + 5% FCS were incubated for 8 hr at 37°C in a humidified 5% CO2, 95% air incubator in the presence of the inducing agents, thymopoietin I, ubiquitin, or medium as a control. After incubation, cell viability was 90%–95%. Cells were then washed once with RPMI-PS and resuspended to 5 x 10^6/ml.

Complement receptor assay. The complement receptor assay was performed according to the technique of Ross et al.13 Briefly, erythrocyte antibody (EA) were made by mixing 1 ml of 5% (v/v) SRBC with 1 ml of 19S rabbit anti-SRBC (1/400) and incubating at 37°C for 30 min. After three washes, erythrocyte antibody complement (EAC) were made by mixing 0.5 ml 5% EA with 0.05 ml of AKR mouse serum as a complement source and incubating at 37°C for 30 min. After three washes both EA and EAC were resuspended to 2 x 10^6 cells/ml. From each fraction of bone marrow 50 µl of cells at 3 x 10^6 cells/µl were mixed with 50 µl of EA or EAC and incubated at 37°C for 30 min with gentle agitation. A total of 300 nucleated cells were counted from each tube immediately and were scored as rosette-positive if four or more SRBC were adherent. Under these conditions, cells from all fractions formed less than 1% EA rosettes.

Human erythrocytes and blood granulocytes have been shown to have surface receptors only for complement components C4b or C3b, while blood lymphocytes have receptors for C4b- C3b and C3d. EAC prepared as described above were incubated with erythrocytes, granulocytes, and mononuclear cells from peripheral blood to determine which receptors they adhered to. Rosettes formed on 12.3% ± 3.2% (mean ± SD) of lymphocytes, 2.3% ± 1.5% of granulocytes, and 0.7% ± 0.7% of erythrocytes. This finding demonstrated that these EAC did not have sufficient quantities of C4b or C3b on their surfaces to bind to the very sensitive C4b C3b receptors present on granulocytes, and thus these EAC were functionally specific markers for C3d receptors.

Cytology. Aliquots of cells from each marrow fraction were centrifuged onto glass slides in a cytocentrifuge. Slides were air dried and stained with tetrachrome. The myeloperoxidase activity of the bone marrow cell fractions was determined by the method of Kaplow.14 Briefly, cytocentrifuge preparations were fixed in formol-ethanol for 60 sec, immersed in the benzidine dihydrochloride safranin staining solution for 15 sec, and rinsed in water for 5 sec. The number of positive cells present was counted under oil immersion in three separate fields of 100 cells.

Phagocytosis. Ten µl of 0.2% (v/v) latex particles were mixed with 50 µl of cells at 5 x 10^6/ml and incubated for 1 hr at 37°C in 5% CO2, 95% humidified air. At the end of the incubation, cells were washed three times in RPMI-PS and resuspended to 5 x 10^6 cells/ml, and cytocentrifuge preparations made. The number of cells ingesting latex particles was determined under oil immersion in three fields of 100 cells.

Chemotaxis. Bone marrow cell chemotaxis was assayed using modified Boyden chambers as described by Wilkinson.15 Marrow cells were suspended in Gey’s solution at 10^6 cells/ml and allowed to migrate through filters of 20-µm pore size for 3 hr at 37°C. Casein, which is highly chemotactic for blood granulocytes and monocytes, was used at 5 mg/ml in Gey’s solution as the chemotactic attractant. Following incubation the filters were stained with hematoxylin and the number of cells per high power field were counted at various depths (20, 40, 60, 80 µm) in the filter.

RESULTS

Experimental Design

Normal human marrow was subjected to the experimental procedures outlined in Fig. 1. The first stage consisted of separating the heterogeneous mixture of many different cell types, first according to density and then by size. In the second stage, different cell populations were incubated with agents known to affect cell surface marker expression on murine hematopoietic cells. In the last
stage, the different cell populations were assayed for expression of myeloperoxidase activity, complement receptors, chemotaxis, and phagocytosis.

Separation of Bone Marrow into Morphologically Distinct Cell Populations

Centrifugation of peripheral blood cells on an F-H gradient provides a population at the F-H/plasma interface which consists of approximately 95% mononuclear cells. However, due to the lower density of immature hematopoietic cells, centrifugation of bone marrow cells on an F-H gradient provides a mixture of immature myeloid and erythroid cells as well as mononuclear cells.

While F-H centrifugation separates cells primarily according to density, velocity sedimentation separates cells mainly according to size. Figure 2 shows the characteristic distribution of human bone marrow cells after separation by velocity sedimentation. Although the procedure is rather lengthy, the viability of separated cells is usually 90%-95%, with a total cell recovery of 80%-90%.

Figure 3 shows the morphology of cells recovered from several different regions of the velocity sedimentation gradient. A mixture of myeloid precursors and blast forms is found in the fastest sedimenting region I. The first peak (region II) contains a population of band forms and segmented granulocytes. The area between the two peaks (region III) is a mixture of erythroid precursors, monocytes, and large lymphocytes, whereas the second peak (region IV) is a pure population of small- and medium-sized lymphocytes. Table 1 gives the detailed differential of cells in the separated fractions.

The Complement Receptor Is a Differentiation Marker for Human Granulocytes

Figure 4 shows the distribution of EAC rosettes in the separated marrow fractions. This figure demonstrates that fractions which contain predominantly band form and mature segmented granulocytes have the highest percentage of EAC rosettes, while the fractions which consist predominantly of promyelo-
INDUCTION OF GRANULOCYTE DIFFERENTIATION

Fig. 2. Distribution of human nucleated marrow cells separated by velocity sedimentation. (A) Absolute number of nucleated cells recovered per fraction. (B) Morphology of cells as percentage of all cells per fraction.

The Complement Receptor Can Be Induced In Vitro on Immature Granulocytes

In experiments using marrow from four different donors ubiquitin and thrombopoietin (0.5 μg/ml) induced an average of 9.3% ± 5.0% (mean ± SE) and 6.8% ± 1.7%, respectively, of the cells in the fraction of immature granulocytes to form EAC rosettes. These agents had an inhibitory effect on complement receptor expression by cells in the mature granulocyte fraction. While this repre-
Fig. 3. Morphology of human marrow cells separated by velocity sedimentation. (A) 7.8 mm/hr. (B) 5.0 mm/hr. (C) 3.5 mm/hr. (D) 2.1 mm/hr.

Presented the mean percent induction in several experiments, ubiquitin showed higher activity than thymopoietin in several experiments where cells from the same donor were used. In addition, incubation times of as little as 2 hr have resulted in the induction of complement receptors in the immature granulocyte fraction. Two additional experiments were performed to determine the dose-
Table 1. Distribution of Human Marrow Cells Separated by Velocity Sedimentation

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Sedimentation Velocity (mm/hr)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td>Myeloid Cells</td>
<td></td>
</tr>
<tr>
<td>Segmented granulocytes</td>
<td>10</td>
</tr>
<tr>
<td>Band forms</td>
<td>19</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>23</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>27</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>13</td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
</tr>
<tr>
<td>Erythroid Cells</td>
<td></td>
</tr>
<tr>
<td>Normoblasts</td>
<td>4</td>
</tr>
<tr>
<td>Erythroblasts</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
</tr>
<tr>
<td>Mononuclear Cells</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
</tr>
</tbody>
</table>

Human marrow was separated first by Ficoll-Hypaque centrifugation and then by velocity sedimentation. Results are expressed as percentage of total nucleated cells present in each fraction of distribution shown in Fig. 2.

response relationship for this phenomenon. The results are shown in Table 2. The peak inducing activity of ubiquitin and thymopoietin occurred at 0.1 μg/ml. By multiplying the number of cells actually induced in each velocity sedimentation fraction by the percentage of original marrow cells found in that fraction, we estimated the percentage of cells inducible for complement receptors in the original bone marrow cell suspension to be 0.5%-2.0%.

An increase in the percentage of cells forming EAC rosettes following incubation with thymopoietin or ubiquitin might simply be due to a selective toxicity of these agents for nonrosetting cells rather than to an inductive effect. Consequently, an aliquot of cells from fractions incubated with medium as a con-
Table 2. Induction of EAC rosettes on immature granulocytes

<table>
<thead>
<tr>
<th>Inducer (ng/ml)</th>
<th>0</th>
<th>10</th>
<th>100</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymopoietin</td>
<td>2.0 ± 0.0</td>
<td>5.3 ± 0.3</td>
<td>8.7 ± 0.3*</td>
<td>4.3 ± 2.3</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>4.0 ± 2.0</td>
<td>4.7 ± 0.7</td>
<td>11.0 ± 2.5†</td>
<td>6.7 ± 0.3</td>
</tr>
</tbody>
</table>

Dose-response relationship for induction of complement receptors by thymopoietin or ubiquitin. Differences between control and induced fractions were analyzed by Student’s t test.

*p < 0.01.
†p < 0.05.

trol, thymopoietin, and ubiquitin was mixed with trypan blue and the absolute number of viable cells was counted in a hemocytometer. Table 3 shows that there was no significant difference in the recovery of viable cells following incubation with medium, thymopoietin, or ubiquitin. The increased percentage of rosetting cells in these experiments, and the increased percentage of cells expressing the other markers in the following experiments was thus due to the induction of marker expression by these agents rather than the elimination of one subpopulation of cells.

Lymphocyte Complement Receptors Are Not Induced in this System

Figure 4 demonstrates that very few of the lymphocytes found in human bone marrow have complement receptors. We have found only 2%-9% EAC-rosetting cells in fractions containing exclusively small- and medium-size lymphocytes. In addition, we have not been able to demonstrate induction of complement receptors in vitro in those fractions with thymopoietin or ubiquitin.

Myeloperoxidase Activity Is Not Induced in this System

The distribution of marrow cells containing myeloperoxidase is shown in Fig. 5. Over 90% of the cells sedimenting 7-9 mm/hr are peroxidase-positive and there is no increase in the number of positive cells following incubation with thymopoietin or ubiquitin. The small percentage of negative cells corresponds to the number of erythroid precursors present in region I. Thus, all of the myeloid cells present in these fractions, even the most immature ones, already express this marker.

Table 3. Recovery of Viable Cells After Incubation

<table>
<thead>
<tr>
<th>Velocity Sedimentation Fractions</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82 ± 9</td>
<td>85 ± 2</td>
<td>91 ± 3</td>
<td>84 ± 7</td>
</tr>
<tr>
<td>Thymopoietin (100 ng/ml)</td>
<td>76 ± 8</td>
<td>90 ± 5</td>
<td>86 ± 3</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>Control</td>
<td>87 ± 12</td>
<td>81 ± 5</td>
<td>82 ± 9</td>
<td>81 ± 8</td>
</tr>
<tr>
<td>Ubiquitin (100 ng/ml)</td>
<td>89 ± 4</td>
<td>83 ± 7</td>
<td>73 ± 9</td>
<td>80 ± 3</td>
</tr>
</tbody>
</table>

Percentage of viable cells recovered following incubation with thymopoietin, ubiquitin, or medium for 8 hr at 37°C. Viability was determined by trypan blue exclusion and data calculated as (number of viable cells before incubation)/(number of viable cells after incubation). Mean ± SE of three experiments is shown. Differences between control and induced fractions were analyzed by Student’s t test. p > 0.1 for all fractions.
Ubiquitin Induces the Capacity to Respond to Chemotactic Agents

The ability of marrow cells to respond to a chemoattractant was determined by measuring the distance migrated through a filter towards the chemoattractant, casein. Figure 6 shows the number of cells in each velocity sedimentation fraction which migrated to a depth of 80 μm. Very few of the cells in the control bone marrow fractions migrated to this depth. Following incubation with ubiquitin, however, a significant increase in the number of cells migrating toward the chemoattractant was observed in the fraction containing cells sedimenting 5.7–6.6 mm/hr (region II), which consists of predominantly band form granulocytes.

It was of importance to determine whether the increased migration of the cells in fraction II was due to an increase in random movement (chemokinesis)
or to an increase in directional locomotion (chemotaxis). Cells in region II which had been incubated as control or with thymopoietin or ubiquitin were placed in Boyden chambers containing casein either below or both above and below the filter. If ubiquitin induced only an increase in random locomotion, an enhanced migration would be seen in both cases. On the other hand, if it induced the ability of cells to respond to a chemotactic agent, then an increased migration would not be observed when the chemoattractant was both above and below the cells, since no concentration gradient would exist. The results are shown in Fig. 7. Ubiquitin clearly induced an increased chemotactic response and not an increase in random locomotion.

**Ubiquitin and Thymopoietin Induce the Capacity to Phagocytose**

The distribution of phagocytic cells in the separated marrow fractions is shown in Fig. 8. This distribution closely parallels that of mature granulocytes.
Table 4. Induction of Phagocytosis by Immature Granulocytes

<table>
<thead>
<tr>
<th>Inducer (ng/ml)</th>
<th>Phagocytic Cells (%)</th>
<th>0</th>
<th>10</th>
<th>100</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymopoietin</td>
<td>28.3 ± 1.2</td>
<td>24.3</td>
<td>± 0.6</td>
<td>37.3</td>
<td>3.2*</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>28.3 ± 1.2</td>
<td>34.7</td>
<td>± 4.6</td>
<td>35.7</td>
<td>± 8.0</td>
</tr>
</tbody>
</table>

Dose–response relationship for induction of phagocytosis by thymopoietin or ubiquitin. Cells tested were in velocity sedimentation region I. Mean ± SE of triplicate measurements are shown. Differences between control and induced reactions were analyzed by Student’s t test.

* p < 0.01.

in marrow. Following incubation with ubiquitin and thymopoietin, a dose-related increase in the number of cells in region I capable of phagocytosing latex particles was observed (Table 4).

DISCUSSION

The study of hematopoietic differentiation in vitro is complicated by several factors. When heterogeneous cell populations are studied, it is not always possible to define clearly the role of cellular interactions or to identify precisely the cells which are involved in a specific differentiative event. We believe the two-step cell-separation system described here provides fractions of human bone marrow sufficiently homogeneous to obtain this kind of information. Similarly, the use of conditioned media or tissue extracts may complicate the dissection of individual stages of induction and regulation of differentiation. We have therefore used two purified polypeptides, thymopoietin and ubiquitin, as tools for identifying cells which respond to a defined stimulus with the induction of a specific stage in differentiation.

The effect of thymic and nonthymic agents on lymphocyte differentiation antigens is an active topic of investigation. We have shown here that thymopoietin and ubiquitin at concentrations as low as $2 \times 10^{-8} M$ can induce the expression of a differentiation marker for granulocytes, the receptor for the complement component C3d. One might question the induction of a receptor which is not even expressed on mature peripheral blood granulocytes. It is certainly not unique for a surface marker to appear at one stage of differentiation and disappear at another. For example, the alloantigen TL is not present on mouse prothymocytes in bone marrow or spleen, is expressed on thymocytes, and disappears when these cells leave the thymus to become mature T lymphocytes. The induction of C3d receptors on cells in the immature granulocyte fraction and inhibition of C3d receptor expression on cells in the more mature granulocyte fraction may be an example in vitro of a similar phenomenon in the sequence of human hematopoietic differentiation.

Induction of complement receptors on immature granulocytes has been demonstrated in other systems. Lotem and Sachs have demonstrated that steroids and DNA synthesis inhibitors induced both complement receptors and more mature morphology of some clones of murine myeloid leukemias. Furthermore, Rabellino and Metcalf have shown that complement receptors are expressed by the progeny of granulocyte colony forming cells (CFU-c) only after 4 days of culture in soft agar, a time at which most cells are myelocytes or
metamyelocytes. Recent studies by Ross et al. involving a density separation of marrow granulocytes confirmed our finding that complement receptors are first expressed on differentiating myeloid cells at the metamyelocyte or band stage and are then lost as the cells become segmented granulocytes. Thus, a variety of different agents have the capacity of inducing in vitro some granulocyte differentiation markers in mice as well as in humans.

Whether the induction in vitro of surface markers is an example of "true differentiation" or only a kind of temporary membrane reorganization or modulation represents a fundamental question. To call surface marker changes true differentiation, one would expect to see some associated functional change. Our studies suggest that this may be happening, but it remains to be established that the induction of phagocytosis or chemotactic response and complement receptors occurs in the same cell.

These experiments also focus attention on the specificity of action of thymopoietin. Scheid et al. have shown in the mouse system that thymopoietin specifically induces the appearance of the T-cell differentiation markers (Thy 1, TL, Ly 1,2,3) but not the B-cell markers (Fc, C3 receptor, Pc 1), while ubiquitin can induce both classes of markers. Thymopoietin appears to have a separate receptor on lymphocyte precursors since the action of ubiquitin but not thymopoietin can be blocked by &-adrenergic blockers.

Recent studies by Hammerling et al. have shown that thymopoietin at higher concentrations can indeed induce the alloantigen Ia and surface immunoglobulin on mouse B cells. Our studies now demonstrate the induction of granulocyte differentiation markers by thymopoietin. That several different cell types should have receptors for this molecule is not surprising, especially in view of the fact that the initial activity of thymopoietin has been demonstrated on neuromuscular receptors. It may be that the specificity of thymopoietin resides not only in the selective expression on cells of its receptor, but also in the restricted distribution of the molecule in vivo. Thus, thymopoietin may be a specific inducer of T-cell differentiation in vivo if it only reaches active concentrations within the thymus. Our preliminary studies on the effect of thymopoietin on human marrow lymphocyte differentiation in vitro suggest that this inducer does exhibit specificity for the induction of some T-cell and not some B-cell markers.

At present, the role of thymopoietin and ubiquitin in normal human granulocyte differentiation in vivo is unclear. It would seem that thymopoietin is not an absolute requirement for granulocyte differentiation since patients with thymic aplasia have been reported to have normal granulocyte numbers and function. Similarly, nude mice which have a genetic lack of thymus do generate mature granulocytes. Whether ubiquitin is a physiologic inducer of human granulocyte differentiation may be determined when techniques for the direct measurement of serum ubiquitin levels have been developed.

Whatever the role of thymopoietin and ubiquitin in vivo, our studies suggest that they can serve as useful tools for the study in vitro of cellular and humoral factors regulating granulocyte differentiation. Studies using these inducers may provide insight into certain diseases in which granulocyte differentiation is blocked. We are now using the in vitro induction of complement receptors by
ubiquitin as an assay for the presence of myeloid progenitors in the marrow of patients with disorders of hematopoietic differentiation such as aplastic anemia, agranulocytosis, congenital neutropenia, and cyclic neutropenia to attempt to identify the pathogenetic mechanisms involved in these diseases.

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Induction of human granulocyte differentiation in vitro by ubiquitin and thymopoietin

WA Kagan, GJ O'Neill, GS Incefy, G Goldstein and RA Good