Expression of Transcobalamin II Receptors by Human Leukemia K562 and HL-60 Cells

By Tatsuhiko Amagasaki, Ralph Green, and Donald W. Jacobsen

Plasma membrane receptors for the serum cobalamin-binding protein transcobalamin II (TCII) were identified on human leukemia K562 and HL-60 cells using immunoaffinity-purified human TCII labeled with [57Co]cyanocobalamin. The Bmax values for TCII receptors on proliferating K562 and HL-60 cells were 4,500 and 2,700 per cell, respectively. Corresponding dissociation constants (Kd) were 8.0 x 10^-11 mol/L and 9.0 x 10^-11 mol/L. Rabbit TCII also bound to K562 and HL-60 cells but with slightly reduced affinities.

Calcium was required for the binding of transcobalamin II to K562 cells. Brief treatment of these cells with trypsin resulted in almost total loss of surface binding activity. After removal of trypsin, surface receptors for TCII slowly reappeared, reaching pretreatment densities only after 24 hours. Reappearance of receptors was blocked by cycloheximide. TCII receptor densities on K562 and HL-60 cells correlated inversely with the concentration of cobalamin in the culture medium. This suggests that intracellular stores of cobalamin may affect the expression of transcobalamin receptors. Nonproliferating stationary-phase K562 cells had low TCII receptor densities (less than 1,200 receptors/cell). However, the density of TCII receptors increased substantially when cells were subcultured in fresh medium. Up-regulation of receptor expression coincided with increased 3H-thymidine incorporation, which preceded the resumption of cellular proliferation as measured by cell density. In the presence of cytosine arabinoside, which induces erythroid differentiation, K562 cells down-regulated expression of TCII receptors. When HL-60 cells were subcultured in fresh medium containing dimethylsulfoxide to induce granulocytic differentiation, the up-regulation of TCII receptors was suppressed. This event occurred well before a diminution of 3H-thymidine incorporation and cessation of proliferation. Thus, changes in the regulation of expression of TCII receptors correlate with both the proliferative and differentiation status of cells.

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Transcobalamin II (TCII) is a serum protein that avidly binds cobalamin (Cbl; B12) and facilitates the cellular uptake of the vitamin throughout the body. The protein, which has little or no carbohydrate and a molecular weight of 43,000, promotes uptake of Cbl in a variety of human and animal cells, including HeLa cells, Ehrlich ascites cells, human fibroblasts, L1210 murine leukemia cells, and liver cells. Although a number of reports indicate the presence of specific receptors for TCII on a variety of mammalian cells, studies on human hematopoietic cells, including leukemia cells, have been limited and have been performed using serum rather than purified TCII as the source of the binding protein. In this study we used purified human and rabbit TCII radiolabeled with [57Co]cyanocobalamin (CN-Cbl) to demonstrate specific receptors for TCII on K562 and HL-60 cells and to characterize their expression during proliferation and differentiation.

MATERIALS AND METHODS

Cell lines. Cells were cultured at 37°C in a humidified atmosphere of 7.5% CO2-92.5% air. K562 cells, a human erythroleukemia cell line, were grown in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 5.0 mg/mL bovine serum albumin (BSA) (A9706; Sigma Chemical Co, St Louis, MO), 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL fungizone (Irvine Scientific, Santa Ana, CA). The CN-Cbl concentration in this medium was 5,000 pg/mL (3.7 x 10^-11 mol/L). The BSA used in the medium had a low but significant unsaturated Cbl binding capacity (UCBC) based on a differential assay designed to detect the presence of TCII. Thus, solutions (10 mg/mL) of BSA had UCBC values of 74 to 114 pg/mL in the absence of monocyanocobinamide (factor B) and 71 to 95 pg/mL in its presence. These results suggest that there may be a small but significant level of bovine TCII (<2 x 10^-12 mol/L) in the K562 culture medium. The concentration of CN-Cbl in RPMI 1640 medium (3.7 x 10^-11 mol/L) is sufficient to saturate the UCBC contributed by the BSA. HL-60 cells, a human myeloid leukemia cell line, were cultured in RPMI 1640 supplemented with 20% fetal calf serum (FCS; HyClone, Logan, VT). The TCII concentration in this medium (contributed by FCS) was approximately 9 x 10^-11 mol/L and was presumably all in the holo-form due to the relatively high concentration of free CN-Cbl in RPMI 1640.

Preparation of [57Co]CN-Cbl. Streptomyces griseus (ATCC 10137) was grown in medium containing 5CoCl2 (carrier-free; Amersham, Arlington Heights, IL) to late-log phase, harvested by centrifugation, and extracted with 0.10 mol/L sodium acetate (pH 4.6) containing 1.0 mmol/L potassium cyanide at 95°C. Extracted [57Co]CN-Cbl was purified by sequential column chromatography on XAD-2 (Serva Biochemicals, Westbury, NY), QAE-Sephadex (Sigma), and SP-Sephadex (Sigma) (details to be published elsewhere). Preparations of [57Co]CN-Cbl were radiochemically pure as judged by high-performance liquid chromatography and had specific activities ranging from 80 to 96 µCi/µg CN-Cbl. The retention times of this preparation and commercially available

From the Department of Laboratory Hematology, Division of Laboratory Medicine, and Department of Brain and Vascular Research, The Research Institute, The Cleveland Clinic Foundation, Cleveland, OH.

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Address reprint requests to Donald W. Jacobsen, PhD, Department of Laboratory Hematology, The Cleveland Clinic Foundation, One Clinic Center, 9500 Euclid Ave, L30, Cleveland, OH 44195-5139.

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[13Co]CN-Cbl (Amersham) were identical to that of authentic CN-Cbl (Sigma).

Preparation of [13Co]CN-Cbl-TCII. TCII was purified to homogeneity from rabbit serum by affinity-photodissociative chromatography as described by Jacobsen and Huennekens,8 and used both as a source of unlabeled TCII to determine nonspecific binding and as an antigen in the preparation of anti-TCII antibodies. Goat antirabbit TCII antisera was obtained after subcutaneous immunization with 165 µg of TCII in complete Freund’s adjuvant followed by booster immunizations with 250 µg of TCII in incomplete Freund’s adjuvant every 4 weeks. The immunoglobulin (Ig) fraction was isolated by ammonium sulfate precipitation at 0 to 2°C as follows: 150 mL of goat antirabbit TCII plasma was treated with 74 mL of saturated ammonium sulfate for 60 minutes with constant stirring. The precipitate was pelleted by centrifugation and dissolved in phosphate-buffered saline (PBS) to a final volume of 50 mL. The solution was then treated with 25 mL of saturated ammonium sulfate for 30 minutes to reprecipitate the Ig fraction. The precipitate was pelleted by centrifugation, redissolved in PBS, and twice reprecipitated with saturated ammonium sulfate. The final pellet was dissolved in PBS and stored at −70°C. Before agaroase coupling, the Ig preparation was dialyzed against PBS at 4°C for 6 hours to remove traces of ammonium sulfate. Goat antirabbit TCII Ig (24.8 mg) was coupled to 50 mL of CNBr-activated agaroase (Sepharose 4BCL, Sigma) by the method of March et al.20 Human serum apo-TCII, the concentration of which had been determined previously,12,16 was radiolabeled with an equivalent amount of commercial [13Co]CN-Cbl (220 µCi/µg CN-Cbl: Amersham) at 23°C for 60 minutes. Rabbit serum apo-TCII was radiolabeled with an equivalent amount of our [13Co]CN-Cbl in similar fashion. [13Co]CN-Cbl-TCII was partially purified from serum by immunosaffinity chromatography as follows: 10 mL of [13Co]CN-Cbl-labeled rabbit or human serum was treated with 1.0 mL of agaroase-antit-CII-antibody for 3 hours at 37°C. The agaroase beads were pelleted by centrifugation and washed three times with 0.05 mol/L potassium phosphate (pH 7.0) containing 0.5 mol/L NaCl followed by three washes with PBS. The beads were stored in PBS at 0°C for up to 1 month. [13Co]CN-Cbl-TCII was released from the beads immediately before use as follows: 0.10 mL of agarose-antibody [13Co]CN-Cbl-TCII beads were suspended in 0.90 mL of 3.0 mol/L sodium thiocyanate and incubated at 0°C for 20 minutes. After centrifugation, BSA was added to the supernatant (final concentration, 1.0 mg/mL), which was dialyzed against 0.01 mol/L tris-HCl (pH 8.0) containing 1.0 mol/L NaCl and 1.0 mg/mL BSA at 4°C for 6 hours. This was followed by overnight dialysis against PBS containing 1.0 mg/mL BSA at 4°C. Each preparation of solubilized [13Co]CN-Cbl-TCII was stored at 0 to 2°C and used within 1 week. After correction for endogenous serum TCII content,11 the specific activities for rabbit [13Co]CN-Cbl-TCII ranged from 30 to 50 dpm/fmol TCII, and for human [13Co]CN-Cbl-TCII the specific activity was 575 dpm/mmol TCII.

Binding assay for TCII receptors. Cells (1 to 3 x 10⁶) were suspended in Hanks’ balanced salt solution (HBSS, Gibco) containing 1 mg/mL BSA and incubated with the desired concentration of rabbit or human [13Co]CN-Cbl-TCII for 60 minutes at 4°C. After incubation, cells were washed three times with HBSS containing 1.0 mg/mL BSA and radioactivity was measured in a gamma spectrometer. The amount of [13Co]CN-Cbl-TCII used for routine binding assays was 25 ng/mL (0.58 nmol/L).

Tritiated-thymidine uptake studies. The incorporation of [3H]-thymidine (H-T; 45 Ci/mmol; ICN, Costa Mesa, CA) into DNA by K562 and HL-60 cells during growth and differentiation was determined as follows: stationary-phase cells were subcultured into fresh medium at initial cell densities of 3 to 5 x 10⁵ cells/mL. K562 cells were subcultured in the presence or absence of 3.7 x 10⁻⁷ mol/L cytosine arabinoside (ara-C). HL-60 cells were subcultured in the presence or absence of 1.25% dimethylsulfoxide (DMSO). At 24-hour intervals, aliquots of cells were gently pelleted, counted, and resuspended in original growth medium in 24-well tissue culture plates (1-mL cultures at a density of 5.0 x 10⁵ cells/mL). After 60 minutes of incubation at 37°C in the presence of 1H-T (K562, 2.0 µCi; HL-60, 1.0 µCi), the cells were collected on 25-mm cellulose acetate filters (type HA, 0.45 µm; Millipore Corp, Bedford, MA), washed two times with 2 mL of 10% trichloroacetic acid, and two times with 1 mL of 95% ethanol. The filters were then dried and radioactivity was determined in a liquid scintillation counter. Net 1H-T uptake for 5 x 10⁵ cells was determined after subtraction of nonspecific membrane binding (less than 1%).

Other methods. Protein was determined by the method of Smith et al22 using a commercial reagent (Pierce Chemical Corp, Rockford, IL). Cell densities were determined by counting with a hemacytometer. Cell viability was determined by trypan blue dye exclusion. The concentration of holo-TCII in serum was determined by radioisotope dilution assay (SimulTRAC Radioassay, Beckon Dickinson, Orangeburg, NY) before and after treatment with microcrystalline silica21 (QUO G-761; PQ Corp, Valley Forge, PA). Hemoglobin-positive K562 cells were determined by the method of Rowley et al.23 Dimethylsulfoxide-induced differentiation of HL-60 cells to granulocytes was monitored by the method of Collins et al.24

RESULTS

Binding of [13Co]CN-Cbl-TCII by K562 and HL-60 cells. Proliferating K562 and HL-60 cells express receptors for TCII. As shown in Fig 1, [13Co]CN-Cbl-TCII from either human or rabbit serum binds to K562 and HL-60 cells in a saturable fashion. Binding of rabbit [13Co]CN-Cbl-TCII to K562 (Fig 1B) and HL-60 (Fig 1D) cells was almost completely blocked by addition of 100-fold molar excess of unlabeled rabbit TCII, indicating extremely low nonspecific binding. Maximum receptor number (Bmax) was slightly higher for rabbit than for human TCII in both cell lines (4,700 v 4,500 receptors/cell for K562 and 3,100 v 2,700 receptors/cell for HL-60). However, this slight difference in receptor content was not statistically significant. The affinity of receptors for human TCII was greater than for rabbit TCII: for K562, the kd for human TCII was 8.0 x 10⁻¹⁰ mol/L compared with 4.2 x 10⁻¹⁰ mol/L for rabbit TCII; for HL-60, the kd for human TCII was 9.0 x 10⁻¹¹ mol/L compared with 3.0 x 10⁻¹⁰ mol/L for rabbit TCII. Rabbit [13Co]CN-Cbl-TCII was used in subsequent studies to characterize the regulation of expression of TCII receptors by K562 and HL-60 cells because of its low nonspecific binding (see Fig 1, B and D) and greater availability.

Calcium dependency. Previous reports have demonstrated that calcium is required for the binding of TCII to its receptors.5,6,8 In the present study, TCII binding by K562 cells was reduced in calcium-free HBSS and almost entirely abrogated with calcium-free HBSS containing 2.0 nmol/L ethylenenbis(oxyethylenenitri1o)tetracetic acid (EGTA) (Table 1). At this concentration of EGTA, cell viability was unaffected as determined by trypan blue dye exclusion. These findings clearly demonstrate that in K562 cells TCII binding to its receptor is calcium-dependent. The dependency of calcium for TCII binding by HL-60 cells was not determined.
Effect of trypsin on TCII receptor density. Exposure of cells to trypsin 250 μg/mL for 20 minutes at 37°C resulted in a near total loss of $^{57}$Co-CN-Cbl-TCII binding by K562 cells. However, when these cells were transferred into trypsin-free medium, TCII receptors reappeared in a time-dependent manner (Fig 2). After 24 hours in culture, the density of TCII receptors returned to pretreatment levels. The rate of reappearance of TCII receptors, which remained linear over the 24-hour period, was approximately 200 receptors/cell/h. Cycloheximide inhibited reappearance of TCII receptors on K562 cells suggesting that de novo protein synthesis was responsible for the reappearance of TCII receptors after their removal by trypsin.

Cell viability was unaffected by the concentration of cycloheximide (2.0 μg/mL) used during the course of this study.

Influence of K562 proliferative status on TCII receptor density. K562 cells that were allowed to grow to high cell densities (stationary-phase) had low levels of TCII receptors. However, when these cells were subcultured into fresh medium at low cell density, TCII receptor number increased markedly, as shown in Fig 3. Cell number did not increase until 24 hours after subculturing. However, the density of TCII receptors began to increase within 15 hours after subculturing. TCII receptor density continued to increase and reached its zenith 48 hours after subculturing. Receptor density then began to decrease at 72 hours, reaching a nadir at 144 hours. In separate studies designed to measure $^3$H-T incorporation during growth, it was found that freshly subcultured K562 cells from stationary-phase cultures had a
Fig 3. Expression of TCI1 receptors during the growth of K562 cells. Stationary-phase K562 cells (3.5 x 10⁶ cells/mL) were subcultured in fresh culture medium at the concentration of 5.0 x 10⁶ cells/mL. At the indicated times, cell density (O) and [⁶⁷Co]CN-Cbl-TCII binding (#) were determined as described in Materials and Methods. The data shown are representative of three separate experiments. Except for a single receptor assay at 144 hours, each point represents a mean value (±SD) of triplicate assays.

low level of incorporation (Table 2). After 24 hours, ³H-T uptake increased nearly 10-fold and reached its peak at 48 hours. This was followed by a rapid decrease in ³H-T uptake as cells approached stationary-phase. Thus, expression of TCI1 receptors by K562 closely parallels the synthesis of DNA in these cells.

Down-regulation of TCI1 receptor expression during ara-C-induced differentiation of K562 cells. K562 cells differentiate to hemoglobin-producing cells when cultured in the presence of low concentrations of ara-C. In the absence of ara-C, less than 1% of cultured K562 cells contain hemoglobin as detected by benzidine staining. After 48 hours of culturing in the presence of 3.6 x 10⁻⁷ mol/L ara-C (viability greater than 95%), 44% of the cells were hemoglobin-positive (Fig 4). During ara-C-induced differentiation, TCI1 receptor density decreased to 38% of the control level after 48 hours. In separate studies the effect of ara-C on ³H-T uptake was determined. As shown in Table 2, 3.6 x 10⁻⁷ mol/L ara-C caused an abrupt decrease in incorporation of ³H-T by K562 cells. At 24 hours ara-C-treated cells incorporated only 2% of the level of ³H-T when compared with nontreated controls. After 48 hours in ara-C, the incorporation was less than 0.5%. Cessation of proliferation in response to ara-C thus resulted in a rapid down-regulation of TCI1 receptors on K562 cells.

Table 2. ³H-Thymidine Uptake by K562 Cells in the Presence and Absence of Ara-C

<table>
<thead>
<tr>
<th>Culture Time (h)</th>
<th>Cell Density (x 10⁶/mL)</th>
<th>³H-T Uptake (dpm x 10⁻⁴)</th>
<th>% Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>3.4</td>
<td>3.2</td>
<td>7.3 (0.43)</td>
</tr>
<tr>
<td>24</td>
<td>4.3</td>
<td>3.3</td>
<td>65.3 (2.7)</td>
</tr>
<tr>
<td>48</td>
<td>7.6</td>
<td>3.2</td>
<td>70.8 (3.0)</td>
</tr>
<tr>
<td>72</td>
<td>9.9</td>
<td>3.3</td>
<td>21.6 (0.87)</td>
</tr>
<tr>
<td>96</td>
<td>19.2</td>
<td>3.3</td>
<td>20.2 (0.69)</td>
</tr>
<tr>
<td>120</td>
<td>29.7</td>
<td>3.3</td>
<td>16.8 (3.7)</td>
</tr>
<tr>
<td>144</td>
<td>32.0</td>
<td>3.6</td>
<td>20.1 (2.4)</td>
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Stationary-phase cells (5 x 10⁶ cells/mL) were harvested and subcultured in fresh medium at an initial cell density of 3.4 x 10⁶ cells/mL. At the times indicated cells were removed for density determinations, ³H-T uptake studies and viability determinations as described in Materials and Methods. ³H-T uptake is for 5 x 10⁶ cells. The mean (±SD) for triplicate determinations are shown. Ara-C concentration was 3.6 x 10⁻⁷ mol/L.
unaffected at this concentration of DMSO. In this experiment, stationary-phase HL-60 cells were subcultured in fresh medium in the presence or absence of DMSO. TCII receptor density of control cells (− DMSO) rapidly increased during the first 24 hours in subculture. In contrast, TCII receptor density increased only slightly in DMSO-treated HL-60 cells during this time (19% of the increase seen in untreated cells). Cellular proliferation rates were identical during this period. In separate experiments, uptake of \( ^3 \text{H}-\text{T} \) by DMSO-treated and -untreated HL-60 cells was similar after 24 hours (data not shown). During the next 24 hours, the TCII receptor density of control cells continued to increase, albeit at a slower rate, whereas receptor density and \( ^3 \text{H}-\text{T} \) uptake in DMSO-treated cells decreased. At 96 hours, \( ^3 \text{H}-\text{T} \) uptake in DMSO-treated cells was 53% of that in untreated cells. Granulocyte differentiation in DMSO-treated HL-60 cells was demonstrated by phorbol myristate acetate-induced phagocytosis and superoxide anion generation followed by nitroblue tetrazolium dye reduction.\(^{24}\) After 96 hours in the presence of DMSO, approximately 35% of the cells contained formazan deposits in contrast to only 6% of the untreated cells. These findings demonstrate that differentiating HL-60 cells suppress up-regulation of TCII receptor expression even though their proliferative status closely parallels that of control cells.

**Effect of Cbl concentration in culture medium on expression of TCII receptors.** To determine the effect of Cbl concentration in the culture medium on expression of TCII receptors by human leukemia cell lines, K562 and HL-60 cells were grown in serum-free and Cbl-free medium.\(^{25}\) Based on Scatchard plot analysis of saturation binding studies (data not shown), the TCII receptor densities on K562 and HL-60 cells grown in the absence of Cbl increased substantially in comparison with cells grown under routine culture conditions (Table 3). The dissociation constants (kd) did not change significantly.

**DISCUSSION**

In contrast to the extensive literature on the expression of transferrin receptors, particularly by K562 cells, there is scant information on the regulation of expression of TCII receptors by human leukemia cells. We undertook this study to characterize the expression of TCII receptors in two established human leukemia cell lines, K562 and HL-60. Specifically, we wanted to determine whether or not these cells regulate the expression of their TCII receptors under controlled conditions of proliferation and differentiation in culture.

The availability of highly purified rabbit TCII and high-specific activity \([^{57}\text{Co}]\text{CN-Cbl-TCII}\) from human and rabbit serum has allowed us to determine accurately TCII receptor densities on K562 and HL-60 cells. Agarose-immobilized goat antirabbit TCII antibody was used to partially purify \([^{57}\text{Co}]\text{CN-Cbl-TCII}\) from human and rabbit serum. Dose-dependent binding studies on proliferating K562 and HL-60 cells showed that both rabbit and human \([^{57}\text{Co}]\text{CN-Cbl-TCII}\) were bound in a saturable manner. K562 cells contain approximately 4,500 TCII receptors/cell, which is considerably less than the number of transferrin receptors (approxim-
TCII RECEPTOR EXPRESSION ON K562 AND HL-60 CELLS

...mately 200,000/cell) found on this cell line. It has been reported that human fibroblasts contain 3,000 to 4,000 TCII receptors/cell. The dissociation constants for human TCII binding to K562 and HL-60 cells were $8 \times 10^{-11}$ and $9 \times 10^{-11}$ mol/L, respectively. The values are similar to the dissociation constants reported for TCII-binding to human fibroblasts ($5 \times 10^{-11}$ mol/L), placenta ($1.8 \times 10^{-11}$ mol/L), and a hepatoma cell line ($3 \times 10^{-10}$ mol/L).

The cellular uptake of CN-Cbl-TCII is a two-phase process involving an initial rapid calcium-dependent and energy-independent step, followed by a much slower secondary phase that is both energy- and temperature-dependent. The latter step is performed by adsorptive endocytosis and involves clathrin-coated pits and vesicles. Although a detailed kinetic analysis of TCII uptake by human leukemia cells was beyond the scope of this work, our data demonstrate specific binding of [55Co]CN-Cbl-TCII to receptors in K562 and HL-60 and that in K562, calcium is essential for this binding.

Our data clearly show that K562 and HL-60 cells regulate the expression of TCII receptors during proliferation and differentiation. Nonproliferating stationary-phase cells express low levels of receptors. In these studies proliferative status was determined by direct cell counting and $^3$H-T uptake. The viability of cells was determined in all experiments by trypan blue dye exclusion. Stationary-phase cells were not used in experimental studies unless the viability exceeded 95%. Stimulation of cell proliferation by subculturing at low density in fresh medium initiates up-regulation of TCII receptor expression and $^3$H-T uptake in both cell lines. As cells approach stationary-phase, the expression of TCII receptors is down-regulated and there is a decrease in $^3$H-T incorporation. Similar observations have been reported for the transferrin receptor. Mitogen-stimulated peripheral blood lymphocytes incorporated considerably more [55Co]CN-Cbl than their unstimulated controls. Additional evidence published by Hall suggests that the up-regulation of TCII receptors on mitogen-stimulated lymphocytes coincides with the most active phase of DNA synthesis. More recently these observations were extended to include human fibroblasts, an Epstein-Barr virus immortalized human lymphoblast cell line (RPMI 6410) and a human hepatoma cell line (HepG2).

To further investigate the apparent correlation between cellular proliferation and expression of TCII receptors, we studied the regulation of receptor expression during induced differentiation of K562 and HL-60 cells. When proliferating K562 cells were treated with ara-C, there was a dramatic reduction in the number of TCII receptors within 24 hours that correlated with a cessation of cell proliferation and $^3$H-T uptake. As K562 cells differentiated (monitored by the number of hemoglobin-positive cells observed), the TCII receptor number decreased further. It should be noted that the initial rapid decrease in TCII receptors preceded the appearance of hemoglobin-positive cells. Thus, down-regulation of TCII receptors is an early event in the differentiation process.

The induction of differentiation by DMSO in HL-60 cells suppresses up-regulation of TCII receptor expression. In these studies, nonproliferating stationary-phase HL-60 cells were subcultured in fresh medium in the presence or absence of DMSO at low cell density. Untreated cells showed rapid up-regulation of TCII receptor expression during the first 24 hours. In contrast, DMSO-treated cells showed only a slight increase in TCII receptor number during the same time period. Receptor number then decreased as the rate of proliferation and $^3$H-T uptake declined in DMSO-treated cells. In contrast to K562 cells, which rapidly ceased proliferation when induced to differentiate by ara-C, HL-60 cells continued to proliferate at a normal rate when stimulated to differentiate by DMSO. Gill and Wickramasinghe reported that uptake of serum-bound [55Co]CN-Cbl progressively decreased during DMSO-induced differentiation of HL-60 cells. Similar results have been obtained for the expression of transferrin receptors in DMSO-induced HL-60 cells.

Factors that control the level of expression of TCII receptors and the intracellular concentration of Cbl have not been well characterized. The expression of transferrin receptors is inversely related to intracellular ferritin iron stores. The amount of Cbl in the medium available to K562 and HL-60 cells may play a role in regulating the level of expression of TCII receptors. Thus, K562 and HL-60 cells grown in medium devoid of TCII and CN-Cbl have higher TCII receptor densities than cells grown in the presence of CN-Cbl and/or serum TCII. It is also possible that K562 and HL-60 cells synthesize and secrete TCII to the culture medium. A sensitive radioimmunoassay for measuring TCII in media and media concentrates is being established to determine this possibility. Secreted TCII could function as an autocrine modulator in controlling the expression of TCII receptors. However, low levels of endogenously produced TCII, if present in culture medium, would not affect the results reported herein because the cells used in these studies were washed before the measurement of ligand binding.

Our data suggest that TCII receptor expression is regulated in part by the availability of Cbl and/or holo-TCII in the culture medium, but more critically by the proliferative and differentiation status of the cell. The regulation of expression of TCII receptors in K562 and HL-60 cells is linked to their proliferative status. Induction of differentiation in K562 cells results in a rapid down-regulation of TCII receptor expression, which coincides with the cessation of cell proliferation. In the case of HL-60 cells, induction of differentiation suppresses the up-regulation of TCII receptor expression well before cellular proliferation ceases.

ADDENDUM

After the submission of this work, a report by Lindemans et al. appeared which describes the effect of DMSO- and 1,25-dihydroxyvitamin D$_3$-induced differentiation on TCII receptor expression in HL-60 cells. These investigators also observed a rapid decline in TCII-mediated surface binding and internalization after induction of differentiation which preceded the cessation of cellular proliferation.

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