Cytotoxic Activity of an Anti-Transferrin Receptor Immunotoxin on Normal and Leukemic Human Hematopoietic Progenitors

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ABSTRACT

The process of cellular iron uptake involves a specific receptor for the plasma carrier transferrin and a pathway of receptor-mediated endocytosis. Transferin receptor expression is closely related to the rate of cell proliferation, and conjugates between anti-transferrin receptor monoclonal antibodies and toxins have been shown to have potent cytotoxic activity. We have constructed an anti-transferrin receptor immunotoxin by conjugating the anti-transferrin receptor monoclonal antibody B3/25 to a ribosome-inactivating protein, the saporin-6 (SO6), which is derived from the seeds of the plant Saponaria officinalis. The immunotoxin B3/25-SO6 was tested for in vitro cytotoxic activity against the human cell lines K-562 and HL-60 and against normal human bone marrow hematopoietic progenitors and acute myeloid leukemia clonogenic cells. The immunotoxin proved to be an effective inhibitor of K-562 and HL-60 clonogenic cell growth, in vitro colony formation being completely inhibited at immunotoxin concentrations ranging from 10^{-11} to 10^{-8} M. B3/25-SO6 markedly reduced the cloning efficiency of 111 (ill clonogenic cellsat 10^{-12}M. Exposure of 111 (ill) cells in suspension culture to 10^{-12} M B3/25-SO6 for 72 h resulted in only 50% suppression of their clonogenic potential. Finally, B3/25-SO6 was found to be a potent inhibitor of in vitro growth of acute myeloid leukemia clonogenic cells. The cytotoxic effects of B3/25-SO6 were shown to be specific, since both saporin alone and irrelevant immunotoxins did not have any effect in the cellular systems examined. We conclude that the immunotoxin B3/25-SO6 has dose-related cytotoxic effects on both normal and leukemic human hematopoietic progenitors. Since there are substantial differences between normal and leukemic progenitors with respect to the proportion of cycling cells and the expression of transferrin receptors, B3/25-SO6 or similar immunotoxins may have clinical application in bone marrow-purging procedures.

INTRODUCTION

Iron is required for cell growth and multiplication in view of its role in the activity of ribonucleotide reductase, a key enzyme in DNA synthesis (1). The process of cellular iron uptake involves a specific receptor for the plasma carrier transferrin and a pathway of receptor-mediated endocytosis (2). Several findings indicate that transferrin receptor expression is related not only to cellular iron requirements for specific metabolic functions but also to the proliferative status of the cell per se (3). The transferrin receptor number increases from late G_i to early S phase of the cell cycle, indicating a close linkage with initiation of DNA synthesis (3).

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In a recent review, Talele (4) analyzed the role of transferrin receptors in hematopoietic cell growth and the possible use of these surface molecules as a target for antileukemia therapy. The use transferrin receptor as a target to introduce drugs or toxins into proliferating cells was first proposed by Trowbridge and Domingo (5) and subsequently investigated by several groups (3). In a previous report (6), we showed that transferrin acquires cytotoxic properties against the K-562 cell line when conjugated with the ribosome-inactivating protein SO6, a toxin derived from the plant Saponaria officinalis which inhibits protein synthesis. These properties were shown to be related to cellular internalization of the conjugated toxin via the receptor for transferrin (6).

In this work, we have developed an anti-transferrin receptor immunotoxin by conjugating SO6 with the monoclonal antibody B3/25, which does not block transferrin receptor function (7). The cytotoxic activity of this immunotoxin was evaluated on human leukemia cell lines, normal human hematopoietic progenitors (CFU-GM and BFU-E), and clonogenic cells of AML. The results obtained indicate that this immunotoxin is effective in killing leukemic clonogenic cells and this and other anti-transferrin receptor immunotoxins might have clinical application in bone marrow-purging procedures.

MATERIALS AND METHODS

Purification of the Vegetal Toxin SO6. The ribosome-inactivating protein SO6 was purified from the seeds of S. officinalis as previously described (8). Its purity was evaluated as described by Lappi et al. (9).

Preparation of Human Transferrin. Transferrin was isolated from human plasma according to the method of Morgan et al. (10). The absorbance ratio A_630/A_280 of the protein at the end of the purification procedure was found to be 0.046, which is typical of pure human transferrin. Purity was also assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis which showed a single band corresponding to a M, 80,000.

The Monoclonal B3/25. The monoclonal B3/25 anti-transferrin receptor (a murine IgG1) (7) was a generous gift of Dr. I. S. Trowbridge.

Construction of Immunotoxins. Transferrin and B3/25 were coupled to SO6 via disulfide bonds using the agent N-succinimidyl 3-(2-pyridyldithio)propionate as described in detail before (6). In the final conjugation reactions 2.5 and 3.5 molecules of SO6 were present for each transferrin or B3/25 molecule, respectively. Protein species (conjugated and unconjugated proteins) from transferrin conjugation were then separated by chromatography on a Mono Q column as previously described (6). B3/25 conjugates were separated by ion exchange chromatography on a Mono S fast-performance liquid chromatography column equilibrated with 20 mM acetate buffer, pH 5.0, and eluted with a concentration gradient of 0–0.5 M NaCl in the same buffer. Eluates from the chromatographic separations were collected in 0.5-ml frac-
tions, and fractions containing transferrin or the antibody, conjugated with one or two molecules of SO6, were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, pooled, dialyzed overnight against phosphate buffered saline, pH 7.2, and used in our experiments. Conjugate concentrations were assayed by enzyme-linked immunosorbent assay.

Clonal Cultures of Leukemic Cell Lines. The K-562 (erythroid) and HL-60 (myeloid) leukemic cell lines were used in our studies. Cells were maintained in exponential growth by suspension culture in RPMI-1640 medium (GIBCO, Grand Island, NY) supplemented with 1-glutamine, antibiotics, and 10% FCS.

The cytotoxic activity of the immunotoxins was evaluated in clonogenic assays. K-562 and HL-60 cells were studied for their cloning efficiency in semisolid medium. In these experiments 300–1000 cells were seeded in 1 ml of 0.3% agar, 15% FCS, and RPMI-1640 medium, and colony growth was evaluated at 10 days. To study the effects of the immunotoxin on the self-renewal capacity of leukemic clonogenic cells, recloning experiments were carried out using 0.9% methycellulose as a semisolid medium. A primary clonogenic assay was performed in the presence or absence of immunotoxin (10^{-12} M). After 10 days, colonies were recovered from methycellulose cultures, pooled, washed, and replated to give an estimate of secondary cloning efficiency.

**In Vitro Colony Assays for Normal Human Hematopoietic Progenitors.** The effects of the immunotoxin B3/25-SO6 were evaluated also on the in vitro growth of normal human bone marrow hematopoietic progenitors of the myeloid (CFU-GM) and erythroid (BFU-E) lineages. Ten hematologically normal subjects gave their informed consent for these studies. LDBMCs were separated by centrifugation on a Ficoll-Hypaque gradient (density 1.077 g/ml) at 400 x g for 40 min at 20°C. Interface cells were washed three times in RPMI-1640 medium and suspended in IMDM (Seromed, Berlin, Federal Republic of Germany) (11, 12).

In the CFU-GM assay, 10^5 LDBMCs were cultured in 1 ml aliquots of 0.3% agar, 15% FCS, IMDM, and 10% conditioned medium from the 5637 bladder carcinoma cell line in 35-mm Petri dishes at 37°C in a 5% CO_2 humidified atmosphere (11). Colonies, aggregates with >40 cells, were scored at day 7 (day-7 CFU-GM) and day 14 of culture (day-14 CFU-GM).

BFU-E (erythroid bursts with >500 cells) were evaluated following culture of marrow mononuclear cells in IMDM, 0.9% methycellulose, 30% FCS, 5% phytohemagglutinin-stimulated leukocyte-conditioned medium, 5 x 10^{-3} M 2-mercaptoethanol (Sigma Chemical Co, St. Louis, MO), and 1 unit human recombinant erythropoietin. Cells, 10^3, were plated in 1-ml aliquots of the medium at 37°C in a humidified atmosphere of 5% CO_2; BFU-E were scored at 14 days.

**CFU-AML Assay.** Bone marrow specimens were obtained by aspiration from patients with AML at clinical onset following informed consent. AML blasts were separated by centrifugation on a Ficoll-Hypaque gradient (density 1.077 g/ml) at 1500 rpm for 20 min at 4°C. After separation, cells were washed three times in RPMI-1640 and suspended in IMDM.

CFU-AML assay was performed by plating in duplicate 5 x 10^4 LDBMCs in 35-mm Petri dishes in 1-ml aliquots of IMDM containing 30% human fresh frozen plasma (from a single donor), 10% 5637-conditioned medium (as a source of colony-stimulating activity), 5 x 10^{-3} M 2-mercaptoethanol, 2 mM glutamine, and 0.9% methycellulose. After incubation for 14 days at 37°C in a humidified atmosphere supplemented with 5% CO_2, cultures were examined by an inverted microscope and the number of AML blast colonies was scored. AML blast colonies were defined as aggregates of >20 undifferentiated blast cells. Representative colonies from each patient were picked up from methycellulose by aspiration, disaggregated in medium, centrifuged onto glass slides, and evaluated for morphology by May-Grünwald-Giemsa stain. The cells in all colonies examined were morphologically leukemic blast cells; at least one colony from each dish was evaluated for morphology by May-Grünwald-Giemsa stain.

**Specificity of the Immunotoxin Cytotoxic Activity.** To evaluate what specific and nonspecific activities could be expected from B3/25-SO6, we evaluated the degree of cytotoxicity to normal and leukemic clonal leukemia cells caused by two irrelevant SO6 immunotoxins, both generous gifts from Dr. D. A. Lappi, Farmitalia-Carlo Erba, Milan, Italy. The first one was made by conjugating SO6 with a monoclonal antibody reactive with the immunoglobulin heavy chain (TEG IgM, an IgG1 mouse monoclonal antibody) (13). The second one was made by conjugating SO6 with the anti-human T-lymphocyte (CD5) monoclonal antibody OKT1 (a murine IgG1) (14). Both monoclonal antibodies were murine IgG1, as was the monoclonal B3/25. The two immunotoxins were evaluated for possible cytotoxicity on in vitro growth of HL-60 clonogenic cells, CFU-GM, and BFU-E as previously described. Similar studies using B-cell restricted immunotoxins were carried out previously by Bregni et al. (15).

**RESULTS**

**K-562 and HL-60 Cell Lines.** In a first set of experiments, we studied the effects of the monoclonal antibody B3/25 and of the immunotoxins B3/25-SO6 and transferrin-SO6 on the in vitro growth of clonogenic cells from the K-562 human leukemic cell line. SO6 alone did not have any inhibitory effect on in vitro growth of K-562 clonogenic cells at concentrations equal to 10^{-7} M or less (data not shown). As shown in Table 1, the monoclonal antibody B3/25 was slightly inhibitory at concentrations ranging from 10^{-7} to 10^{-9} M; only values for clonogenic cell growth at 10^{-7} M, however, were significantly different from control (t = 7.07, P < 0.05). On the contrary, both SO6 conjugates were potent inhibitors of colony formation: the monoclonal antibody-toxin conjugate completely suppressed clonogenic cell growth in a range from 10^{-7} to 10^{-11} M and appeared more suppressive than the transferrin-toxin conjugate at the lower concentration tested (10^{-12} M, Table 1).

The B3/25-SO6 immunotoxin also was found to be a potent inhibitor of the HL-60 clonogenic cells, completely suppressing colony formation in a concentration range from 10^{-7} to 10^{-11} M (Table 2). The monoclonal antibody B3/25 alone was inhibitory only at 10^{-7} M (t = 8.98, P < 0.05). When we examined the cloning efficiency of HL-60 cells that had been pretreated for different periods in suspension culture, washed, and then plated for colony formation, it became apparent that pretreatment of cells for 1–72 h with 10^{-9} M immunotoxin progressively impaired their clonogenic activity (F = 231.02, P < 0.00001, Fig. 1) This was consistent with a cytotoxic activity of the immunotoxin; it also showed that complete killing of HL-60

| Table 1 Effects of anti-transferrin receptor monoclonal antibody B3/25, B3/25-SO6 immunotoxin, and transferrin-SO6 conjugate on the in vitro growth of clonogenic cells from the K-562 human leukemic cell line (n = 3) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **K-562 colonies (no./dish)** |
| **Molarity** | **B3/25** | **B3/25-SO6** | **Transferrin-SO6** |
| Control | 30 ± 5 | 30 ± 5 | 30 ± 5 |
| 10^{-7} | 15 ± 8 | 0 | 0 |
| 10^{-8} | 19 ± 11 | 0 | 0 |
| 10^{-9} | 20 ± 9 | 0 | 0 |
| 10^{-10} | 25 ± 16 | 0 | 0 |
| 10^{-11} | 28 ± 3 | 0 | 10 ± 4 |
| 10^{-12} | 31 ± 6 | 3 ± 1 | 23 ± 8 |

* Mean ± 1 SD.
clonogenic cells required a fairly protracted exposure (≥48 h).

We took advantage of the high plating efficiency of the HL-60 cells (30–100%) and carried out cloning experiments to evaluate the effects of the immunotoxin on the secondary clonogenic potential and, consequently, the self-renewal capacity of clonogenic leukemic cells. In these studies, the immunotoxin was used at very low concentration (10⁻¹² M) in order to obtain high replating efficiency and, consequently, the self-renewal capacity of the immunotoxin.

**Normal Human Hematopoietic Progenitor Cells.** Bone marrow erythroid colony growth was studied in 5 normal subjects (Table 4). The immunotoxin completely inhibited BFU-E growth at high concentrations (10⁻⁸-10⁻⁹ M), and the suppressive effect was progressively lost at lower concentrations (10⁻¹⁰-10⁻¹³ M). Analysis of variance showed that concentration of the immunotoxin B3/25-SO6 significantly influenced BFU-E growth (F = 16.27, P < 0.00001).

Bone marrow CFU-GM growth was studied in 10 normal subjects. As shown in Table 5, the immunotoxin markedly inhibited colony formation at concentrations up to 10⁻¹⁰ M. Analysis of variance showed that concentrations of the immunotoxin B3/25-SO6 significantly influenced both day-7 CFU-GM (F = 7.77, P < 0.00001) and day-14 CFU-GM (F = 9.09, P < 0.00001) growth. There were no significant differences between day-7 and day-14 CFU-GM in growth inhibition.

The relationship between duration of exposure to the immunotoxin B3/25-SO6 and degree of clonogenic cell killing was studied on normal day-14 CFU-GM. LDBMCs were cultured for 1–72 h at 37°C in a 5% CO₂ humidified atmosphere in 6-well tissue culture plates at a concentration of 1 x 10⁶/ml in RPMI-1640 supplemented with 10% heat-inactivated FCS, 5 x 10⁻³ M 2-mercaptoethanol, 2 mM glutamine, and antibiotics, in the presence or absence of 10⁻⁹ M immunotoxin. At the end of the pretreatment period, LDBMCs were washed and then plated for evaluation of day-14 CFU-GM growth. There was no substantial cell death due to culture since in the control the number of vital cells after 72 h was 97 ± 11% of the initial value. The results of these experiments are reported in Fig. 2. Although exposure to B3/25-SO6 for increasing periods significantly reduced day-14 CFU-GM growth (F = 6.13, P < 0.01), 50% of these hematopoietic progenitors retained their clonogenic potential after 72 h of exposure.

**CFU-AML.** The immunotoxin B3/25-SO6 also was found to inhibit CFU-AML growth. In the 10 patients studied, 4 had no in vitro leukemic colony formation; the results concerning the remaining 6 patients are reported in Table 6. Leukemic clonogenic cell growth was completely inhibited at concentrations of 10⁻⁸-10⁻⁹ M and markedly decreased at 10⁻¹⁰ M.

**Immunotoxin Specificity.** As shown in Table 7, the two irrelevant immunotoxins tested (TEC-SO6 and OKT1-SO6) did not show any significant cytotoxicity on the in vitro growth of HL-
Culture, washed, and then plated for evaluation of day-14 CFU-GM growth. Cells were pretreated with 10^{-8} M immunotoxin for different periods in suspension culture, then plated for evaluation of day-14 CFU-GM growth.

- 6.13, P < 0.01), but about 50% of these hematopoietic progenitors retained their clonogenic potential after 12 h exposure. Marrow cells for 1-72 h progressively impaired CFU-GM clonogenic activity (F = 6.13, P < 0.01), but about 50% of these hematopoietic progenitors retained their clonogenic potential after 72 h exposure.

For each patient, colony scores at different concentrations are reported. No basal colony growth was observed in patients 1, 4, 6, and 10.

Table 6 Effects of various concentrations of B3/25-SO6 on the in vitro growth of normal bone marrow day-14 CFU-GM, and BFU-E (n = 3)

<table>
<thead>
<tr>
<th>CFU-GM (no./dish)</th>
<th>B3/25-SO6 molarity</th>
<th>2^a</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24</td>
<td>7</td>
<td>18</td>
<td>9</td>
<td>43</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>10^{-8}</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10^{-9}</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10^{-10}</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10^{-11}</td>
<td>12</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>15</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>10^{-12}</td>
<td>18</td>
<td>5</td>
<td>13</td>
<td>6</td>
<td>28</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

a Patient number.

Table 7 Effects of two irrelevant SO6 immunotoxins on the in vitro growth of HL-60 clonogenic cells, normal human bone marrow day-14 CFU-GM, and BFU-E (n = 3)

<table>
<thead>
<tr>
<th>ImmunoToxin</th>
<th>HL-60 colonies (no./dish)</th>
<th>Day-14 CFU-GM (no./dish)</th>
<th>BFU-E (no./dish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>204 ± 54^a</td>
<td>37 ± 13</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>TEC/SO6 (10^{-8} m)</td>
<td>211 ± 70</td>
<td>35 ± 15</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>OKT1/SO6 (10^{-8} m)</td>
<td>224 ± 49</td>
<td>41 ± 17</td>
<td>22 ± 8</td>
</tr>
</tbody>
</table>

Mean ± 1 SD.

60 clonogenic cells and of normal human bone marrow day-14 CFU-GM and BFU-E.

DISCUSSION

Several studies have been carried out in recent years to explore the potential for manipulations of cellular iron metabolism in modulating normal and malignant cell proliferation (3, 4). Part of these studies has been based on the use of transferrin receptor as a cell surface target for monoclonal antibodies or immunotoxins. Three features make the transferrin receptor a potential target for agents to modulate cell proliferation (16): (a) it is involved in a process of receptor-mediated endocytosis (2); (b) its expression is closely linked to the proliferative status of the cell, more precisely the initiation of DNA synthesis (3); (c) it is abundantly expressed on some tumor cells (17).

Attempts to target the transferrin receptor have been mainly based on the use of monoclonal antibodies conjugated with plant or bacterial toxins (16, 18-20). Most plant toxins are ribosome-inactivating proteins and behave as potent inhibitors of protein synthesis so that delivery of only a few molecules of immunotoxin to the cytoplasm is sufficient to kill the cell (21). Saporin has been purified from the seeds of S. officinalis and is one of the most active ribosome-inactivating proteins against isolated ribosomes or acellular systems (21). Like some of these vegetal toxins, saporin is a single-chain polypeptide unable to bind the cell surface structures and enter the cell, so that in its native form it is only mildly toxic, or nontoxic, for intact cells.

For example, when unbound SO6 was tested against normal human hematopoietic progenitors (22), only high concentrations (10^{-6}-10^{-7} M) were found to be inhibitory, while no cytotoxic effect was observed at lower concentrations (10^{-5} M or less). In our preliminary experiments, we did not find any inhibitory effect at concentrations equal to 10^{-7} M or less.

Bound to monoclonal antibodies against transferrin receptor, saporin and similar toxins can be transported into the cell via receptor-mediated endocytosis, inhibit protein synthesis, and affect the cell growth (23). In this study, we have shown that the B3/25-SO6 immunotoxin has potent cytotoxic activity against clonogenic cells from both leukemia cell lines and AML patients. Since the most important parameter of inhibition of leukemic colonies is reduction in the number of clonogenic cells within the colonies (24), we have carried out sequential clonal cultures. This approach allowed us to demonstrate that the immunotoxin suppressed leukemic stem cell generation (Table 3). Furthermore, exposure of leukemic clonogenic cells to the immunotoxin for 48-72 h in suspension culture completely abolished their clonogenic potential (Fig. 1). Being clonogenic cells responsible for malignant progression, this suppressive effect appears particularly useful.

From a clinical point of view, the questions are what specific and nonspecific activities can be expected from the B3/25-SO6 immunotoxin, and, in particular, whether its potent cytotoxic activity against leukemic clonogenic cells may have clinical application in bone marrow-purging procedures. Our data, in fact, indicate that B3/25-SO6 can inhibit normal BFU-E and CFU-GM growth, the erythroid progenitors being more sensitive to the cytotoxic activity of the immunotoxin than the myeloid ones (Tables 4 and 5). This is consistent with data of Shannon et al. (25) who found that both transferrin receptor expression and transferrin requirement are higher in human BFU-E than in myeloid progenitors.

To address the first question, i.e., specificity of B3/25-SO6 cytotoxicity against cells expressing transferrin receptors, we have used as isotype-matched negative controls a B-cell- and a T-cell-restricted saporin immunotoxin developed by Lappi et al. (13-15). These immunotoxins were found to have no significant effect on the in vitro growth of normal and leukemic human hematopoietic progenitors (Table 7), as Lappi et al. had previously shown in their own experiments. More recently, Dinota et al. (26) developed a new approach to in vitro bone marrow purging of multidrug-resistant cells based on the use of a mouse monoclonal antibody directed against M, 170,000 glycoprotein and a saporin-conjugated anti-mouse antibody.
Also, this irrelevant saporin immunotoxin was shown to have no effect on human normal hematopoietic progenitors. From all these observations, it can be concluded that saporin immunotoxins are highly specific and, in particular, that the irrelevant ones do not affect the in vitro growth of normal human hematopoietic progenitors. The same is not true for other toxins. For example, Dinota et al. (27) constructed an immunotoxin by conjugating an anti-B-cell monoclonal antibody with the toxin momordin; after exposure of bone marrow cells to 10^{-8} M immunotoxin for 24 four h, day-14 CFU-GM rescue was 48 ± 22%.

The second question to be addressed is whether the immunotoxin is equally cytotoxic against normal hematopoietic progenitors. Based on the above reported data, one would conclude that there is no differential cytotoxic effect of the immunotoxin against normal and leukemic progenitors. This is certainly true as far as progenitor cells expressing transferrin receptors are concerned. However, when we exposed normal hematopoietic progenitors to the immunotoxin for 1–72 h, about 50% of day-14 CFU-GM retained their clonogenic potential (Fig. 2), a pattern substantially different from that of HL-60 clonogenic cells (Fig. 1). This suggests that the percentage of day-14 CFU-GM expressing transferrin receptors was substantially lower than that of HL-60 clonogenic cells. It should be also noted that a 50% progenitor rescue is considered good in bone marrow purging and that the number of CFU-GM is at present the most used index for cytotoxicity to hematopoietic progenitors (26, 27).

Lesley et al. (28) addressed the issue of the toxicity that an anti-transferrin receptor immunotoxin might have on bone marrow hematopoietic progenitor cells. In a murine model, they showed in vitro exposure to an anti-transferrin recepto immunotoxin resulted in the killing of virtually all late CFU-erythroid and a fraction of CFU-myeloid. However, the pluripotent stem cells capable of repopulating the hematopoietic system of lethally irradiated animals were spared. The sensitivity of each progenitor cell population to the anti-transferrin receptor immunotoxin correlated with the proportion of cells in cycle and their expression of transferrin receptors determined by fluorescence-activated cell sorting (28).

With respect to human hematopoietic progenitors, transferrin receptors have been found to be expressed on about 50% of CFU-GM (4) but only weakly expressed on the more immature CFU-granulocyte, erythrocyte, macrophage, megakaryocyte (29). Presumably, they are not expressed at all in hematopoietic stem cells which in steady-state marrow are dormant in the G0 period of the cell cycle (30). The opposite is true for leukemic clonogenic cells. Kozloski et al. (31) studied the expression of transferrin receptors on AML blasts and blast cell progenitors. They sorted leukemic cells into a transferrin receptor-positive and -negative fraction and found that the vast majority of CFU-AML was in the transferrin receptor-positive fraction. These data are consistent with previous observations by Minden et al. (32), indicating that the AML blast cell population is maintained by a small subpopulation of clonogenic cells, most of which are in the S phase of the cycle.

The fact that transferrin receptors are expressed more on leukemic progenitors than on normal ones suggests that transferrin receptor may be a suitable candidate for the specific targeting of cytotoxic therapy in hematological malignancies. Purging of human bone marrow from actively cycling malignant cells in autologous bone marrow transplantation could be a potential application. The relatively long period (48–72 h) required for complete killing of leukemic clonogenic cells in suspension culture should not be considered a limit. Barnett et al. (33) showed, in fact, that bone marrow can be maintained in culture for 7–10 days to serve as an autograft. Moreover, by using hematopoietic growth factors, it is possible to increase the number of leukemic cells in S phase (thus expressing high numbers of transferrin receptors) (34), without recruiting normal hematopoietic stem cells, whose survival in the G0 period of the cell cycle is independent of early hematopoietic regulators (30).

REFERENCES


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