Retinoic acid-induced CD38 antigen as a target for immunotoxin-mediated killing of leukemia cells

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Abstract

A major obstacle in the successful delivery of antibody-based therapeutics to tumor cells is the heterogeneity of target antigen expression. We reported previously that retinoic acid (RA) is a potent and selective inducer of the cell-surface antigen CD38 in myeloid leukemia cells. The purpose of this study was to determine whether the RA-induced CD38 antigen could be a target for an anti-CD38-based immunotoxin to induce selective killing of leukemia cells. The combination of RA and the anti-CD38 gelonin immunotoxin induced a synergistic killing of leukemia cells. Thus, coculture of myeloid leukemia cells and cell lines with as little as 1 nM RA in the presence of immunotoxin induced substantial killing (> 90%) of leukemia cell clones. More importantly, the blasts of myeloid leukemia patients, irrespective of their morphological and phenotypic features, also responded to the RA and immunotoxin combination when cultured ex vivo. A similar synergistic effect between RA and immunotoxin was observed against a multidrug-resistant variant subline of HL-60 cells. However, another variant of HL-60 cells, HL-60R, in which the retinoid receptor function has been abrogated by a trans-dominant-negative mutation, exhibited complete resistance to the immunotoxin-induced killing effect in the presence or absence of RA. Our results suggest that RA combined with anti-CD38-based therapeutic agent may offer exciting opportunities for the treatment of myeloid leukemias despite their multiplicity of genetic and clinical varieties.

Introduction

The use of monoclonal antibodies (mAbs) to deliver drugs or toxins to distinct molecular structures expressed on the surface of unwanted tumor cells represents an attractive and potentially useful strategy for cancer treatment (1). Theoretically, such a targeted approach to therapy could be a major advance in the selective elimination of tumor cells while also reducing the toxicity of treatment toward normal nontargeted tissues. Nevertheless, in practice, many problems exist that need to be addressed before immunotoxin or antibody drug therapies can become truly effective (1, 2).

One of these potential problems that could theoretically limit the success of antibody-based therapies is the heterogeneity of target antigen expression within a tumor cell population. If a few cells within a tumor lacked the target antigen or expressed it only very weakly, then these cells would escape destruction because of a failure of antibody-mediated delivery of the cytotoxic agent to those particular cells. A possible means of overcoming this problem would be to identify agents that induce high levels of cell-surface target molecules, in the expectation that previously antigen-negative tumor cells would express the target molecules in abundance after treatment.

We have observed previously that retinoids in general and retinoic acid (RA) in particular induce high levels of CD38 antigen expression in several myeloid leukemia cells and cell lines (3–9). RA is quite unique and selective in its ability to induce the expression of CD38; at picomolar concentrations, it induces appreciable accumulation of mRNA and protein levels of CD38 in HL-60 cells (5). Moreover, RA-induced increase in CD38 expression is rapid and is not observed in response to other differentiation-inducing agents such as DMSO, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, interferon-γ, and 12-O-tetradecanoylphorbol-13-acetate (5, 6). CD38 antigen is a cell-surface glycoprotein of M, 46,000, the expression of which is predominantly restricted to lineage-committed lymphoid, erythroid, and myeloid precursor cells in the bone marrow. In the lymphoid cell lineage, CD38 expression continues through the early stages of T-cell and B-cell development. Mature resting lymphocytes express undetectable levels of CD38, but the expression is up-regulated during activation and differentiation of B cells into plasma cells (10). Transformed counterparts of normal hematopoietic cells, such as myeloid leukemia, lymphoma, and myeloma cells, express high levels of CD38 antigen. However, because of retinoic acid receptor (RAR) α fusion with acute promyelocytic leukemia (APL) protein caused by the t(15;17) chromosomal translocation, APL cells or M3 cells, according to the French-American-British classification system, express very low or undetectable levels of CD38 antigen (5). Indeed, RA-mediated CD38 expression in myeloid leukemia cells mandates the presence of functional RARα protein (11, 8).

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The purpose of this study was to determine the ability of the RA-induced CD38 antigen to serve as a target for the delivery of the anti-CD38-conjugated plant toxin gelonin to selectively kill leukemia cells. The results obtained suggested that in vitro and ex vivo treatment of myeloid leukemia cells with RA and anti-CD38 immunotoxin synergistically induced killing of leukemia cells and may be a useful tool for selective elimination of leukemia cells from patients.

Materials and Methods

Materials

All-trans-RA was purchased from Sigma-Aldrich (St. Louis, MO). CD336 and CD293 were kindly provided by Dr. Uwe Reichert (CIRD-Galderma, Sophia Antipolis, France). For each compound, a 10 mM stock solution in DMSO was prepared and stored in small aliquots at −80 °C in the dark. The final concentration of DMSO in culture media to deliver RA never exceeded 0.001%. IB4, a murine anti-human CD38 mAb that recognizes an epitope in the extracellular domain of the CD38 antigen, was affinity purified from ascites (12, 9). Phycoerythrin (PE)-conjugated anti-human CD38 mAb (Leu 17) and normal IgG1 were purchased from Becton Dickinson (San Jose, CA).

Cell Culture

The parental clones of HL-60, K562, KG-1, and THP-1 cells were obtained from the American Type Culture Collection (Rockville, MD). A subclone of HL-60 cells resistant to the RA-induced differentiation effect (i.e., HL-60R) and HL-60R cells retrovirally transfected with full-length RARα cDNA were kindly provided by Dr. Steven Collins (Fred Hutchinson Cancer Center, Seattle, WA; Ref. 13). The APL-derived NB4 cell line was a kind gift of Dr. Michel Lanotte (Hôpital St. Louis, Paris, France). The patient-derived lymphoma cell lines Z-138 and Z-33 were established and provided by Dr. Richard Ford (MD Anderson Cancer Center, Houston, TX). All the cell lines were cultured in RPMI 1640, supplemented with 10% fetal bovine serum, streptomycin (100 μg/ml), penicillin (100 units/ml), HEPES (10 mM), and glucose (2 mM), and were maintained in the log phase of cell growth. Leukemia cells from patients with acute myeloid leukemia (AML) were purified by passing the peripheral blood containing 80% or more leukemia blasts through Ficoll-Hypaque as described (5). Leukemia cells were characterized by morphology after May-Grünwald staining and classified according to the French-American-British system (M1–M5). In patients with APL (M3), leukemic cells were further characterized by the t(15;17) chromosomal translocation.

CD38 Antigen Expression

CD38 expression in untreated and RA-treated (10 nM, 18 h, unless otherwise specified) cells was determined by flow cytometry, immunostaining, or RT-PCR analysis. In brief, cells were stained with PE-conjugated anti-CD38 mAb or an isotypic control immunoglobulin (PE-labeled IgG1) as described earlier (6). The fluorescence was then detected on the log scale using a FACScan flow cytometer and Lysis II Research Software (both from Becton Dickinson). CD38 levels were expressed as signal-to-noise (S/N) ratio defined by the mean fluorescence of CD38-expressing cells divided by the mean fluorescence of cells stained with the isotypic control antibody.

For immunostaining, RA-treated and untreated cells were subjected to cytopsin, fixed for 10 min in 4% formalin, and washed with PBS. The slides containing cells were incubated with 5% bovine serum albumin, washed in PBS, and incubated overnight in PBS/1% bovine serum albumin containing the IB4 anti-CD38 mAb. After three washes in PBS, slides were incubated with anti-mouse biotinylated IgG (Vector Laboratories, Inc., Burlingame, CA), washed in PBS, and incubated with FITC-conjugated streptavidin (Zymed, San Francisco, CA). Finally, the slides were washed in PBS and mounted in antifade medium for viewing under the fluorescence microscope.

Similarly, the CD38 mRNA transcript levels in control and RA-treated HL-60 cells were determined by RT-PCR as described (11). Briefly, total RNA isolated from RA-treated or untreated cells was used to synthesize the first-strand cDNA that in turn was amplified by PCR by using synthetic primers designed to amplify the extracellular domain of human CD38 cDNA (770-bp product).

A dot blot containing polyadenylated RNA from multiple human tissues (human multiple expression array; BD Biosciences Clontech, Palo Alto, CA) was hybridized to a randomly primed cDNA probe containing the entire extracellular domain of human CD38 (770 bp; Ref. 11, 8). Random priming was achieved with radio-labeled [α-32p]dCTP. Hybridization was performed at 65 °C for 6 h, and blots were washed with 2× standard saline citrate plus 1% SDS at 60 °C for 40 min. The blots were subjected to autoradiography at −70 °C.

Conjugation of IB4 to Gelonin

Recombinant gelonin (rGel) containing an extra cysteine residue for site-specific conjugation was generated as described previously (14) and conjugated to the IB4 anti-CD38 mAb using N-succinimidyl 3-(2-pyridyldithio)propionate, also as described (15). The immunoon conjugate was purified using fast-protein liquid chromatography system (Pharmacia, New York, NY) combining gel permeation (S-200) and affinity (Blue Sepharose) chromatography. Purity of the immunoon conjugate was assessed by SDS-PAGE and Western blot analysis.

Cytotoxicity Assay

The immunotoxin-induced cellular cytotoxicity was examined using the CellTiter96 Aqueous cell proliferation assay system (Promega, Madison, WI) according to the manufacturer’s instructions. Briefly, quadruplicate samples containing 1.5–2 × 10⁴ cells/well/0.2 ml in 96-well microwell plates were incubated with RA and immunotoxin at appropriate concentrations. Control wells received either RA, immunotoxin, IB4 alone, or rGel plus RA or RA plus rGel and IB4 together. After 72 h treatment, the number of viable cells remaining in the well was determined by measuring their ability to reduce
3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2H-tetrazolium (MTS) into a soluble formazan. The formazan formed was determined by measuring the absorbance at 490 nm using an automatic plate reader (Molecular Devices Co., Sunnyvale, CA). The cell viability was extrapolated from $A_{490}$ values and expressed as percentage survival using the following formula:

$$\text{% Cell viability} = \frac{A_{490} \text{ of treated samples}}{A_{490} \text{ of untreated or control sample}} \times 100$$

In some cases, cells were preincubated overnight with RA and washed prior to their treatment with the immunotoxin.

**Results**

**RA Induces High Levels of CD38 Antigen Expression in Leukemia Cells**

As reported previously (4–11), treatment of myeloid leukemia cells with submicromolar concentrations of RA induced rapid and massive accumulation of the cell-surface CD38 antigen. Figure 1 shows the extent of CD38 expression in HL-60 and KG-1 cells following an 18 h treatment with 10 nM RA. The flow cytometric histograms shown in Fig. 1A demonstrate that very few untreated HL-60 (19.4 ± 12.1%) or KG-1 (12.6 ± 5.3%) cells are CD38 positive (compared with an isotypic control), with a S/N ratio of 5.4 ± 4.1 and 2.1 ± 1.3, respectively. However, in the presence of RA, more than 99% of cells became CD38 positive in both cell lines, with a S/N ratio of 92.8 ± 14.6 and 159.2 ± 24.9, respectively.

Immunofluorescent staining of RA-treated cells strongly supported the flow cytometry data. Untreated HL-60 cells showed no detectable expression of CD38 antigen, whereas RA-treated (10 nM, 18 h) cells showed strong CD38 antigen expression as revealed by their strong immunoreactivity to the IB4 mAb (Fig. 1B). The increase in CD38 expression in response to RA treatment was due to transcriptional regulation of the CD38 gene as suggested by rapid accumulation of the CD38-specific transcript in RA-treated HL-60 cells (Fig. 1C). The RA-mediated induction of the CD38 gene was under the direct control of RAR$, \alpha$; the presence of a 100-fold excess of that RAR$\alpha$ antagonist, CD2503, almost completely blocked the RA-induced CD38 expression (data not shown).

**In Vitro Cytotoxicity of IB4-rGel Immunotoxin**

To determine whether the RA-induced CD38 antigen could serve as a target for delivering the plant toxin gelonin to leukemia cells, a CD38-selective immunotoxin was constructed by linking the single-chain ribosomal inhibitory protein to the anti-CD38 mAb IB4 as described in Materials and Methods. The $M_r$ of IB4-rGel immunotoxin was ~180 kDa (Fig. 2A), demonstrating a 1:1 molar ratio of IB4 and rGel. We used IB4 mAb to deliver the toxin because previous studies have shown that IB4 is a high-affinity mAb and is effectively internalized after its binding to the cell-surface CD38 antigen (16). Moreover, we have demonstrated previously that the chemically derived immunotoxins containing the toxin gelonin are highly stable; at least 50% of the injected immunotoxin could be recovered intact in plasma of mice after 8 h of i.v. administration (17).

Next, we studied the ability of the immunotoxin to kill RA-induced and uninduced HL-60 cells by comparing the ability of the viable cells remaining following treatment with immunotoxin to inhibit formazan formation. As shown in Fig. 2B, incubation with the immunotoxin alone exerted a small but consistent cytotoxic effect against HL-60 cells; the concentration of immunotoxin without RA...
Retinoic Acid Augments Immunotoxin-Mediated Killing

Figure 2. Characterization of anti-CD38-rGel immunotoxin. A, silver-stained PAGE analysis of the IB4-rGel conjugate. Lane 1, native rGel; lane 2, IB4 mAb; lane 3, purified IB4-rGel conjugate. As seen in lane 3, there was no unreacted rGel and only a very small (<10%) amount of unconjugated antibody could be detected in the purified fraction. B, comparison of the cytotoxicity induced by IB4-rGel immunotoxin in the presence (+RA) or absence (-RA) of RA against log-phase HL-60 cells. Cells were plated for 24 h, and increasing amounts of IB4-rGel immunotoxin were added. In a parallel culture, cells were incubated with an equivalent amount of unconjugated rGel and/or IB4 in the presence or absence of RA as controls. Cell viability was determined after 72 h treatment by MTS assay as described in Materials and Methods.

Table 1. RA-induced CD38 antigen as a target for IB4-rGel-induced killing of leukemia cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CD38 expression (S/N ratio)a</th>
<th>IC50 (ng/ml)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–RA</td>
<td>+RA</td>
</tr>
<tr>
<td>HL-60</td>
<td>1.8</td>
<td>93.1</td>
</tr>
<tr>
<td>HL-60R</td>
<td>0.97</td>
<td>1.8</td>
</tr>
<tr>
<td>HL-60R/RAα</td>
<td>1.96</td>
<td>143.9</td>
</tr>
<tr>
<td>HL-60R/RXα</td>
<td>0.98</td>
<td>1.2</td>
</tr>
<tr>
<td>KG-1</td>
<td>1.7</td>
<td>179.4</td>
</tr>
<tr>
<td>NB4</td>
<td>40.8</td>
<td>89.6</td>
</tr>
<tr>
<td>THP-1</td>
<td>23.2</td>
<td>63.8</td>
</tr>
<tr>
<td>Z-138</td>
<td>141.3</td>
<td>152.4</td>
</tr>
<tr>
<td>Z-33</td>
<td>168.1</td>
<td>159.0</td>
</tr>
</tbody>
</table>

aCells were treated overnight with medium alone or medium containing RA (10 nM). At the end of incubation period, cells were analyzed for CD38 antigen expression using flow cytometry and the results were expressed as S/N ratio as described in Materials and Methods.

bCells were treated with increasing concentrations of immunotoxin in the presence or absence of RA. At the end of incubation period, viable cells were determined by MTS assay as described in Materials and Methods and the dose of immunotoxin needed to kill 50% of cells (IC50) was calculated from the cell viability plot.

RA, retinoid X receptor α.

To determine the critical level of CD38 expression needed to induce an optimal immunotoxin-mediated killing effect against the target cells, we incubated HL-60 cells in the presence of increasing concentrations of RA (0.1–12 nM) and a fixed concentration of immunotoxin (0.83 nM). However, simultaneous presence of RA during incubation with the immunotoxin substantially decreased this concentration to ~7 ng/ml (0.04 nm). RA alone (data not shown) or in combination with either unconjugated IB4 or rGel failed to induce any appreciable cytotoxicity against HL-60 cells. A similar effect of RA in augmenting the immunotoxin-induced cytotoxicity was observed against human monocytic (THP-1), myeloblastic leukemia (KG-1), and APL (NB4) cell lines (Table 1). The patient-derived lymphoma cell lines Z-138 and Z-33, which expressed high basal levels of CD38 antigen, were highly responsive to immunotoxin even in the absence of RA (Table 1).

To determine the critical level of CD38 expression needed to induce an optimal immunotoxin-mediated killing effect against the target cells, we incubated HL-60 cells in the presence of increasing concentrations of RA (0.1–12 nM) and a fixed concentration of immunotoxin (20 ng/ml). Results shown in Fig. 3A (inset) clearly suggest that RA at a concentration as small as 2 nM was able to induce a 4–5-fold increase in the cell-surface CD38 expression. These levels were sufficient to produce considerable increase in immunotoxin-induced cytotoxicity against target leukemia cells. Moreover, preincubation of HL-60 cells with RA (10 nM, 18 h), followed by their washing and subsequent incubation with immunotoxin, also augmented the cytotoxic effect, but optimal activity was observed only when RA was present during the incubation with immunotoxin (data not shown).

The synergistic effect of RA on immunotoxin-induced killing of leukemia cells was strictly related to the ability of RA to induce CD38 antigen expression. The presence of free IB4 mAb at an ~100-fold excess completely rescued target cells from RA plus immunotoxin-induced cytotoxicity (Fig. 3B). The observed protective effect of IB4 mAb was primarily related to its ability to compete with the immunotoxin binding to the cell-surface CD38 antigen. However, a small effect could have been attributed due its agonistic properties to induce proliferative response in HL-60 cells, as reported earlier (18). Moreover, CD2503, a RA-specific antagonist that prevents RAα-mediated

Figure 3. IB4-rGel-mediated killing of HL-60 is dependent on RA-induced CD38 antigen expression. A, cells were incubated with increasing concentrations of RA (2–15 nM) in the presence (●) or absence (○) of immunotoxin (50 ng/ml). Seventy-two hours later, cell viability was determined by MTS assay as described in Materials and Methods. Points, averages of eight values from two independent experiments; bars, SD. Inset, levels of CD38 antigen expression on HL-60 cells after 18 h of treatment with increasing concentrations of RA. Results were expressed as S/N ratios extrapolated from the flow cytometry data as described in Materials and Methods. B, HL-60 cells were incubated in the presence of RA (10 nM) with (●) or without (○) immunotoxin (IT, 50 ng/ml) and increasing concentrations of the unconjugated anti-CD38 IB4 mAb. After 72 h incubation, cell viability was determined by MTS assay as described in Materials and Methods.
signaling and transcriptional regulation of the target genes including CD38 (11), reversed the RA-dependent killing effect of the immunotoxin (data not shown). Notably, the HL-60R cells that harbor a trans-dominant-negative mutation in the RARα gene (13) and exhibit complete resistance to RA-induced CD38 antigen expression (Fig. 4A; Ref. 6) failed to respond to immunotoxin-induced cytotoxicity both in the presence and in the absence of RA (Fig. 4B). Retrovirus-mediated transduction of functional RARα in HL-60R cells reconstituted the ability of these cells to express CD38 antigen in response to RA treatment and rendered them sensitive to the immunotoxin-induced cytotoxic effect in the presence of RA (D).

To further evaluate the synergistic effect of RA- and immunotoxin-induced killing of leukemia cells, we tested a multidrug-resistant HL-60 subline, HL-60_Dox, selected by continuous culture of the parental HL-60 cells in the presence of increasing concentrations of doxorubicin. The HL-60_Dox cells exhibited ~350-fold resistance to doxorubicin when compared with the parental HL-60 cells (Fig. 5A). The resistance to doxorubicin was associated with an increased expression of P-glycoprotein (P-gp), a cell-surface protein that plays a role in the exclusion of drugs from inside out (Fig. 5B). However, the presence of RA (10 nM) during culture with immunotoxin rendered both the drug-sensitive and the drug-resistant HL-60 cells almost equally sensitive to the immunotoxin-induced toxic effect (Fig. 5C versus Fig. 2B). Simultaneous presence of both agents was critical for the optimal killing effect; RA alone or immunotoxin alone exerted no or only a moderate killing effect against either of the two HL-60 cell lines.

To study whether RA with immunotoxin could exert similar synergistic effect against patients’ blasts, we treated primary blasts from six AML patients ex vivo with a combination of RA and immunotoxin. A strong cytotoxic effect of immunotoxin was observed against AML blast cells when cultured in the presence of RA (Table 2). As observed with other leukemia cell lines (Table 1), the augmenting effect of RA on immunotoxin-induced cytotoxicity was associated with an increased expression of CD38 antigen. The blast cells from patients with AML (M2) expressed higher basal levels of CD38 antigen and responded well to the immunotoxin-induced killing effect compared with those from patients with APL (M3; Table 2). However, the presence of RA augmented immunotoxin-induced cytotoxic effect against both cell types, although the effect was more pronounced against APL blasts.

Moreover, the multiple tissue array expression containing mRNA from several human tissues revealed that CD38 was predominantly expressed in thymus (Fig. 6, E5). Lower-level expression was detected in the spleen, lymph nodes, and prostate. In all other tissues, CD38 transcript was either absent or barely detectable (Fig. 6). These data suggested that a combination of RA and anti-CD38-blocked immunotoxin could offer an effective and selective approach to eradicate leukemia blasts in AML patients.

**Discussion**

Our study demonstrated that RA-induced CD38 antigen expression could be an effective target for delivering
anti-CD38-rGel immunotoxin. The results obtained suggested that treatment of leukemia cells with RA at a nanomolar concentration could render these cells exquisitely sensitive to the immunotoxin-induced killing effect.

CD38 is a 45-kDa cell-surface protein glycoprotein with a short NH2-terminal cytoplasmic domain and a long COOH-terminal extracellular domain (8, 19). It participates in transduction of cell activation and proliferation signaling pathways, serves as an adhesion molecule for lymphocyte attachment to endothelium via its ligand CD31, and regulates intracellular calcium by exerting ADP ribosyl cyclase activity and catalyzing the synthesis of cyclic ADP-ribose from NAD+ (8, 9, 19, 20). We chose the CD38 antigen as a target for delivering immunotoxin because it is selectively expressed on immature cells of the hematopoietic system as well as on their malignant counterparts; it first appears on CD34-positive committed stem cells and on lineage-committed progenitors of lymphoid, erythroid, and myeloid cells. Normal resting peripheral blood cells expressed undetectable levels of basal CD38 antigen and showed very little or no increase in response to RA treatment suggesting that anti-CD38-blocked immunotoxin should not cause any adverse effects on these cells.

Table 2. RA-induced and basal CD38 antigen expression on AML blasts and response to IB4-rGel immunotoxin-induced killing

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type of blasts</th>
<th>Treatment</th>
<th>CD38a</th>
<th>IC50 (ng/ml)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>% Positive</td>
<td>S/N ratio</td>
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<tr>
<td>1</td>
<td>AML</td>
<td>Immunotoxin only</td>
<td>18.4</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>AML</td>
<td>Immunotoxin only</td>
<td>96.4</td>
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<td>99.8</td>
<td>81.5</td>
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Note: APL and AML cells were incubated with increasing concentrations of the immunotoxin (1 – 100 ng/ml) in the presence or absence of RA (10 nM). After a 72 h incubation, cell viability was determined by MTS assay, and IC50 was calculated from the viability plot.

*aExpression of CD38 in APL and AML cells with and without RA treatment. Results are the mean values from duplicate experiments.

bND, not done.
Thus, the potent killing effect of IB4-rGel against CD38-positive leukemia cells, coupled with the lack of the CD38 antigen on normal blood cells and other tissues, suggested that this immunotoxin might be useful as therapy of CD38-positive malignant cells of AML and multiple myeloma. More important, the use of anti-CD38-based immunotoxins would be expected to have only a transient effect, if any at all, on normal maturing precursor cells in the bone marrow because most primitive pluripotential stem cells of the hematopoietic system are CD38 negative. Indeed, hematopoietic colonies from CD34+/CD38− progenitor cells have been generated, which supports the view that depletion of CD38+ cells may not deplete non-lineage-committed CD34+/CD38− progenitor cells (21, 22). Moreover, in a previous study, the anti-CD38 HB7 mAb-blocked ricin was shown to exert only a limited toxicity against normal progenitors of the bone marrow (23).

In general, antibody conjugates of rGel appear to have better selectivity, better tumor localization, and more substantial therapeutic properties than do immunoconjugates containing other toxins, such as ricin or abrin (24). A recent report by McGarth et al. (25) contradicts this assumption by suggesting that a P-gp-expressing variant of HL-60 cells (RV+) exhibit selective resistance to a M195-rGel. Also of note, the ricin A chain, which inhibits the same downstream RNase target in cells, was effective in killing RV+ cells (25). In contrast to this report, however, we did not see any resistance to the IB4-rGel-mediated killing effect against HL-60DOX cells despite their P-gp expression and high resistance to doxorubicin (Fig. 5). Moreover, the IB4-rGel immunoconjugate is highly cytotoxic to CD38-positive target cells, irrespective of whether they express P-gp. The cytotoxicity appears to be specific for antigen-bearing cells; HL-60R cells that lacked CD38 expression were completely resistant to IB4-rGel (Fig. 4). Conversely, the cells that had high basal expression of CD38 were highly responsive to the cytotoxic effects of the immunotoxin (Table 1).

A major challenge in treating cancer using antibody-based therapies is the heterogeneity of antigen expression on cells within a tumor. To overcome this problem, we used RA, which is highly specific and potent inducer of CD38 in leukemia (Fig. 1) and lymphoma cells (5–7), in anticipation that leukemia cells with low or absent levels of CD38 antigen could be induced to express high levels of the antigen for effective delivery of the immunotoxin. The cytotoxicity induced by IB4-rGel alone was 3–5 logs higher than that induced by rGel alone. Addition of RA (10 nM) to the immunotoxin caused an additional 2 log increase in cell killing by IB4-rGel (Fig. 2). The RARα-selective retinoids were most potent in inducing CD38 expression and sensitizing cells to the immunotoxin-mediated killing effects. Conversely, HL-60R cells that harbored a dominant-negative mutation in the RARα gene, rendering these cells resistant to RA, showed complete resistance to the immunotoxin-induced killing effects in the presence and absence of RA (Fig. 4).

The potent effect of RA on the induction of the cell-surface CD38 antigen, coupled with the specific cytotoxicity of the IB4-rGel, suggested that these agents may have clinical utility for treating myeloid leukemias in which retinoids are known to induce CD38 expression (Table 1). Indeed, our previous results strongly supported this contention and suggest that a single oral dose of RA (45 mg/m²) could induce a 4–7-fold increase in CD38 expression on leukemia blasts from patients with APL (5). Moreover, ex vivo culture of AML blasts with RA plus IB4-rGel was more effective in inducing cytotoxicity than was culture with either agent alone (Table 2). Because CD38 is a hematopoietic lineage-restricted cell-surface antigen, the accessibility of RA plus IB4-rGel to leukemia cells should not be a problem; in contrast, its accessibility to cancer cells in solid tumors is considered problematic because of its high $M_f$.

Besides leukemia, anti-CD38-directed immunotoxin may also have therapeutic potential for treating other conditions that involve eradication of CD38-positive cells such as multiple myeloma and lymphomas. Similarly, the pathogenesis of diseases such as systemic lupus erythematosus and myasthenia gravis, which are characterized by the secretion of self-reactive antibodies by plasma cells expressing high levels of CD38 antigen, can be corrected using this approach. Moreover, the expression of CD38 antigen is up-regulated in activated T lymphocytes; this up-regulation may be a target for eliminating self-reactive T lymphocytes that underlie the pathogenesis of rheumatoid arthritis and other non-antibody-mediated autoimmune diseases.

Figure 6. RNA dot blot analysis for CD38 expression in a human multiple tissue array. Polyadenylated RNA from 76 human tissues and cell lines was hybridized with a radiolabeled cDNA probe specific for CD38. The concentration of mRNA at each dot was adjusted by the supplier (BD Biosciences Clontech) to produce normalized signals for various housekeeping genes, allowing comparison of gene expression. The highest level of CD38 expression was observed in adult thymus tissue (5E), whereas the remaining tissues in the blot displayed either an extremely low (e.g., lymph node (7E1), spleen (4E), fetal thymus (6G), and prostate (7C1)) or undetectable level of CD38 expression.

References


