Anti-CD38–Blocked Ricin: An Immunotoxin for the Treatment of Multiple Myeloma

By Victor S. Goldmacher, Lizabeth A. Bourret, Beth A. Levine, Robert A. Rasmussen, Majid Pourshadi, John M. Lambert, and Kenneth C. Anderson

We report the development of a potent anti-CD38 immunotoxin capable of killing human myeloma and lymphoma cell lines. The immunotoxin is composed of an anti-CD38 antibody HB7 conjugated to a chemically modified ricin molecule wherein the binding sites of the B chain have been blocked by covalent attachment of affinity ligands (blocked ricin). Conjugation of blocked ricin to the HB7 antibody has minimal effect on the apparent affinity of the antibody and no effect on the ribosome-inactivating activity of the ricin A chain moiety. Four to six logs of CD38+ tumor cell line kill was achieved at concentrations of HB7-blocked ricin in the range of 0.1 to 3 nmol/L. Low level of toxicity for normal bone marrow (BM) granulocyte-macrophage colony-forming units (CFU-GM), burst-forming units—erythroid (BFU-E), colony-forming units—granulocyte/erythroid/monocyte/macrophage (CFU-GEMM) was observed. Greater than two logs of CD38+ multiple myeloma cells were depleted from a 10-fold excess of normal BM mononuclear cells (BMMCs) after an exposure to HB7-blocked ricin under conditions (0.3 nmol/L) that were not very toxic for the normal BM precursors. HB7-blocked ricin was tested for its ability to inhibit protein synthesis in fresh patients' multiple myeloma cells and in normal BMMCs isolated from two healthy volunteers; tumor cells from four of five patients were 100-fold to 500-fold more sensitive to the inhibitory effect of HB7-blocked ricin than the normal BM cells. HB7 antibody does not activate normal resting peripheral blood lymphocytes, and HB7-blocked ricin is not cytotoxic toward these cells at concentrations of up to 1 nmol/L. The potent killing of antigen-bearing tumor cells coupled with a lack of effects on peripheral blood T cells or on hematopoietic progenitor cells suggests that HB7-blocked ricin may have clinical utility for the in vivo or in vitro purging of human multiple myeloma cells.

MUTIPLE MYELOMA remains an incurable malignancy with a median survival of only 48 months when conventional therapies are used. Whether combination chemotherapy is superior to melphalan and prednisone remains controversial. The recent use of α2b interferon during initial treatment and/or as a maintenance therapy, and the use of high doses of chemoradiotherapy followed by hematopoietic stem cell transplantation have both been shown promise in some preliminary studies for selected patients with myeloma. However, at present, myeloma remains incurable, and innovative treatment strategies are needed.

Immunotoxins or hormonotoxins formed by conjugation of potent toxins to monoclonal antibodies (MoAbs) or hormones that bind to cell surface molecules on myeloma cells represent a potentially useful novel treatment strategy. For example, based upon the necessary role of interleukin-6 (IL-6) in the growth of myeloma cells and the presence of IL-6 receptors on the surface of such cells, IL-6 has been linked to pseudomonas exotoxin and diphtheria toxin with the hope of using the IL-6 receptor pathway for the delivery of these toxins specifically to myeloma cells. The widespread expression of IL-6 receptors on normal tissues, including hematopoietic stem cells, may limit the potential clinical utility of such toxins. Other antigens on the cell surface of myeloma cells include those present on normal B cells at various stages of differentiation from the pre B to the plasma cell as well as antigens expressed on non-B cells.

To date, an immunotoxin to target myeloma cells has been prepared by conjugating the 8A MoAb, which recognizes both plasma cells and B-cell precursors to the ribosome-inactivating protein momordin. The most strongly and uniformly expressed antigen on cells identified on clonal populations of myeloma cells is CD38. This antigen has been recently identified as nicotinamide adenine dinucleotide (NAD) glycohydras and is expressed in all myelomas, most cases of T- and B-lineage acute lymphoblastic leukemias, some acute myelocytic leukemias, follicular centre cell lymphomas, and T-lymphoblastic lymphomas. Only a few normal cell lineages express CD38: B cells, including pre-pre-B cells, pre-B cells, and plasma cells; T cells, including cortical thymocytes and activated T cells; basophils; and a subpopulation of macrophages. The strong expression of CD38 on myeloma patients' malignant plasma cells compared with its pattern of expression on normal cells suggests that this antigen may be a useful target for the in vivo or in vitro depletion of tumor cells while sparing normal cells. Indeed, a chimeric heteroconjugate using the Fab portion of an anti-CD38 antibody and a human IgG1 fragment has been shown to mediate antibody-dependent cellular cytotoxicity and is undergoing clinical testing.

In this report, we describe the in vitro cytotoxic properties of a conjugate of the anti-CD38 MoAb HB7 with ricin that has been chemically modified so that its galactose-binding sites are blocked by covalently attached affinity ligands. This HB7-blocked ricin conjugate shows a 4- to 6-log depletion of CD38+ malignant plasma cells at concentrations that do not significantly alter the in vitro proliferation of hematopoietic progenitor cells. The potent specific cytotoxicity of this immunotoxin for tumor cells combined with its low cytotoxicity for normal cells suggests that it may be efficacious for either the in vivo or in vitro killing of multiple myeloma cells.

From ImmunoGen, Inc, Cambridge, MA; and the Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, MA.

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Address reprint requests to Victor Goldmacher, ImmunoGen, Inc, 149 Sidney St, Cambridge, MA 02139.

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MATERIALS AND METHODS

Antibody and immunocjugate preparation. The anti-CD38 MoAb HB7 (IgG1) was developed by Dr Max D. Cooper (University of Alabama at Birmingham). The antibody was purified from ascites fluid as described previously.66 Blocked ricin was prepared and conjugated to the HB7 antibody using methods described elsewhere.45,67 Analysis by sodium-dodecyl sulfate polyacrylamide gel electrophoresis showed that the conjugate consists predominantly of a single species of apparent Mr, of 222,500 corresponding to an immunocjugate containing one molecule of blocked ricin (apparent Mr, 62,500) linked to one molecule of antibody (apparent Mr, 160,000), and that the conjugate is free of both unconjugated antibody and blocked ricin (limit of detection, <5%). The ricin A chain from the immunotoxin and from native ricin were equally potent in their ability to inhibit protein synthesis in a cell-free translation system from rabbit reticulocytes.68 The preparation and the cytotoxic properties of anti–CD19-blocked ricin, anti–CD56-blocked ricin and anti–CD6-blocked ricin have been described previously.66,45,69 The MoAb BC-4E8560 (Kabi Pharmacia, Helsingborg, Sweden) was conjugated to blocked ricin in a similar manner.

Cells. The following human cell lines were used: HS-Sultan (plasmacytoma, American Type Culture Collection [ATCC], CRL 1484); Namalwa (Burkitt’s lymphoma, ATCC, CRL 1452); RPMI 8226 (myeloma, ATCC, CCL 155); U266B1 (myeloma, ATCC, TIB 196); Raji (Burkitt’s lymphoma, ATCC, CCL 86); Jurkat (acute T-cell leukemia, ATCC, TIB 152); Daudi (Burkitt’s lymphoma, ATCC, CCL 213); SUDHL-I and SUDHL-10 (diffuse histiocytic lymphoma, ATCC, TIB 152); and Daudi (Burkitt’s lymphoma, ATCC, CRL 160,000). Cell lines were grown in RPMI-1640 medium supplemented with 10% heat-treated fetal bovine serum (FBS) or essential medium (MEM) for suspension cultures supplemented with 10% iron-fortified bovine calf serum and 2 mmol/L L-glutamine, and then plated in 96-well plates in RPMI-1640 medium supplemented with 20%, rather than 10%, heat-inactivated FBS. The antibody was purified from ascites fluid as described previously.61 U-937 (histiocytic lymphoma, ATCC, CRL 1593); Molt-4 (acute lymphoblastic leukemia, ATCC, CRL 1582); HL-60 (promyelocytic leukemia, ATCC, CCL 240); ALL-3 (pre-B–acute lymphoblastic leukemia62); SW2 (small cell lung carcinoma63), and SCaBER (human bladder squamous carcinomna, ATCC, HTB 3). Cell lines were grown in RPMI-1640 medium supplemented with 10% heat-treated fetal bovine serum (FBS) or 10% iron-fortified bovine calf serum and 2 mmol/L L-glutamine, and maintained in exponential growth cultures. Peripheral blood (PB) lymphocytes were isolated by a standard Ficoll-Hypaque technique from blood freshly drawn from healthy donors. Bone marrow (BM) specimens were obtained from healthy volunteers and from multiple myeloma patients in accordance with protocols approved by the Human Subject Protection Committee of the Dana-Farber Cancer Institute. BM mononuclear cells (BMMCs) were isolated using Ficoll-Paque (Pharmacia LKB Biotechnology, Piscataway, NJ) density gradient centrifugation technique.

Cytotoxicity assays. Cytotoxicity tests on cell lines were performed by incubating test samples with cells at 37°C for 24 hours. The cells were then washed and placed into fresh medium for measuring surviving fractions of cells directly by either the growth back- extrapolation assay,64 or a cloning assay65 that has been modified as follows. HS-Sultan myeloma cells [4 x 10⁶ cells per mL with or without 3.6 x 10⁶ γ-irradiated (2,500 cGy)] normal BMMCs per mL] were exposed to HB7-blocked ricin for 24 hours, washed in fresh medium, and then plated in 96-well plates in RPMI-1640 medium supplemented with 20%, rather than 10%, heat-inactivated FBS.

Cytotoxicity testing on PB T cells was done by incubating test samples with resting T cells at 37°C for 24 hours. The cells were then washed and placed into fresh medium containing phytohemagglutinin, and the surviving fractions of cells were measured in a cloning assay using γ-irradiated autologous PB lymphocytes as feeder cells.

Inhibition of protein synthesis by BMMCs from multiple myeloma patients and healthy volunteers and by HS-Sultan and RPMI 8226 cell lines was detected by [3H]-leucine incorporation. In these experi-
was confined to cell lines of B-cell origin. Interestingly, CD38 was expressed more uniformly than CD19 on all tested cell lines of B-cell origin.

The binding of HB7 antibody to the surface of the myeloma lines HS-Sultan, RPMI 8226, and U266B1, as well as the Burkitt’s lymphoma line Namalwa, was studied further. The average numbers of CD38 antigens per cell were determined with a saturating concentration (40 nmol/L) of \(^{[125]}\)-labeled HB7 antibody and were found to be \(2.7 \times 10^3\), \(1.4 \times 10^3\), \(1.0 \times 10^2\), and below the detection limit \((10^3)\) for the cell lines RPMI 8226, HS-Sultan, Namalwa, and U266B1, respectively. As expected, HB7 antibody showed equal avidity towards the CD38 antigen on all cell lines with an apparent \(K_d\) of 1.5 to 3 nmol/L (Fig 1). The avidity of HB7-blocked ricin conjugate towards CD38-expressing cells was found to be about threefold less than the avidity of the nonconjugated antibody (Fig 2).

Cytotoxicity of HB7-blocked ricin for CD38-expressing cultured cell lines. The cytotoxicity of HB7-blocked ricin on CD38-expressing Namalwa cells is shown in Table 2 and Fig 3A. For comparison, the cytotoxic effects of ricin, N901-blocked ricin (N901 is an anti-CD56 antibody; CD56 is not expressed on Namalwa cells), and of HB7-blocked ricin in the presence of an excess of HB7 antibody are also shown. Namalwa cells are very sensitive to the cytotoxic effect of HB7-blocked ricin with an IC\(_{50}\) value of \(7 \times 10^{-12}\) mol/L, which is almost as sensitive as to native ricin (IC\(_{50}\) of \(4.5 \times 10^{-12}\) mol/L). A nonbinding immunotoxin, N901-blocked

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### Table 1. Expression of CD38 and CD19 on the Surface of Various Cell Lines as Detected by Indirect Immunofluorescence Flow Cytometric Analysis With HB7 and Anti-B4 Antibody

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Origin</th>
<th>% CD38+</th>
<th>% CD19+</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS Sultan</td>
<td>Plasmacytoma/IgG multiple myeloma</td>
<td>97</td>
<td>86</td>
</tr>
<tr>
<td>RPMI 8226</td>
<td>light chain secreting myeloma/multiple myeloma</td>
<td>93</td>
<td>3</td>
</tr>
<tr>
<td>Namalwa</td>
<td>Burkitt’s lymphoma</td>
<td>98</td>
<td>93</td>
</tr>
<tr>
<td>ALL3</td>
<td>pre-B–acute lymphoblastic leukemia</td>
<td>94</td>
<td>89</td>
</tr>
<tr>
<td>Raji</td>
<td>Burkitt’s lymphoma</td>
<td>98</td>
<td>89</td>
</tr>
<tr>
<td>Daudi</td>
<td>Burkitt’s lymphoma</td>
<td>99</td>
<td>91</td>
</tr>
<tr>
<td>SUDHL-1</td>
<td>Diffuse histiocytic lymphoma</td>
<td>96</td>
<td>82</td>
</tr>
<tr>
<td>SUDHL-10</td>
<td>Diffuse histiocytic lymphoma</td>
<td>91</td>
<td>91</td>
</tr>
<tr>
<td>U266B1</td>
<td>IgE secreting myeloma/IgE myeloma</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Molt-4</td>
<td>Acute lymphoblastic leukemia</td>
<td>97</td>
<td>1</td>
</tr>
<tr>
<td>Jurkat</td>
<td>Acute T-cell leukemia</td>
<td>71</td>
<td>1</td>
</tr>
<tr>
<td>HL-60</td>
<td>Promyelocytic leukemia</td>
<td>92</td>
<td>1</td>
</tr>
<tr>
<td>U-937</td>
<td>Histiocytic lymphoma</td>
<td>97</td>
<td>5</td>
</tr>
<tr>
<td>SW2</td>
<td>Small cell lung carcinoma</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

### RESULTS

Binding of anti-CD38 antibody HB7 and its blocked ricin conjugate to cells. To examine the expression of CD38 by various cell lines, the binding of a saturating concentration of HB7 antibody (0.1 \(\mu\)mol/L) to these cells was measured by indirect immunofluorescence (Table 1). Most tested lines of B, T, and myeloid/monocytic origin had a high \((\approx 70\%)\) and uniform expression of CD38, with one exception; myeloma cell line U266B1 expressed the antigen only sparingly \((21\%)\) positive cells). In addition, the expression of the B-cell lineage-specific antigen CD19 (antibody anti-B4) was examined (Table 1) to allow subsequent comparative analysis of HB7-blocked ricin and a highly potent anti-B4–blocked ricin. As expected, the expression of CD19
because neither the nonbinding immunotoxin N901-blocked effect of HB7-blocked ricin was entirely antigen-specific. More sensitive to HB7-blocked ricin (IC30 of 6 x 10^-9 mol/L) than Namalwa cells (Fig 3B). HS-Sultan cells were 5-fold sensitive to SW2 cells with an IC30 of 1 x 10^-11 mol/L after a 24-hour exposure (data not shown).

Similar results were obtained with a multiple myeloma cell line HS-Sultan that is 70-fold more resistant to ricin than Namalwa cells (Fig 3B). HS-Sultan cells were fivefold more sensitive to HB7-blocked ricin (IC30 of 6 x 10^-11 mol/L) than to ricin (IC30 of 3 x 10^-10 mol/L). Again, the cytotoxic effect of HB7-blocked ricin was entirely antigen-specific because neither the nonbinding immunotoxin N901-blocked ricin nor HB7-blocked ricin in the presence of an excess of HB7 antibody killed HS-Sultan cells at the concentrations tested. Up to 5 logs (99.999%) of Namalwa or HS-Sultan cells could be eradicated by treatment with concentrations of blocked ricin in the range of 1 x 10^-10 to 8 x 10^-10 mol/L (Fig 3). The effect of CD38 antigen density on the cytotoxicity of HB7-blocked ricin was analyzed using the highly CD38+ multiple myeloma cell line RPMI 8226, and a myeloma cell line U266B1 that only weakly expresses CD38. The data in Table 2 show that RPMI 8226 cells are, as expected, sensitive to HB7-blocked ricin (with an IC50 value of 7 x 10^-11 mol/L), and that U266B1 cells could not be affected with concentrations of HB7-blocked ricin of up to 2 x 10^-9 mol/L (the highest concentration tested). Although the cell surface phenotype of the clonogenic cell in myeloma is unknown, most malignant plasma cells strongly express CD38 in a pattern similar to that observed on RPMI 8226 cells.50

Malignant plasma cell depletion by HB7-blocked ricin in BM samples. Having shown that HB7-blocked ricin is highly potent and selective in killing CD38-expressing cell lines, we next determined whether this immunotoxin would be effective in killing residual malignant cells in a sample of BM. An admixture of 90% freshly isolated BM mononuclear cells and 10% HS-Sultan cells was treated with HB7-blocked ricin, and the surviving myeloma cells were detected in a clonogenic assay. As a control, HS-Sultan cells alone were treated with the immunotoxin under similar conditions (Fig 4). At the concentration of 0.3 nmol/L immunotoxin, more than two logs of these cells were depleted from the BM sample. Importantly, the degree of malignant plasma cell kill was similar in the presence and in the absence of the BM cells.

Effect of HB7-blocked ricin on normal hematopoietic stem cells. The toxicity of HB7-blocked ricin for normal hematopoietic BM clonogenic cells was evaluated to determine the safety of this immunotoxin for the ex vivo purging of tumor cells from BM. BMMCs from normal individuals were either incubated with medium or with medium containing HB7-blocked ricin at various concentrations for 24 hours, and the number of CFU-GM, BFU-E, and CFU-GEMM were then determined (Table 3). HB7-blocked ricin was only moderately toxic for these progenitors with less than 1 log depletion at concentrations (10^-10 to 10^-11 mol/L) that deplete 5 to 6 logs of antigen-bearing tumor cells. This toxicity to hematopoietic stem cells is similar to that of another blocked ricin conjugate with an antibody (anti-CD33) that is not

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Ricin</th>
<th>HB7-Blocked Ricin</th>
<th>N901-Blocked Ricin</th>
<th>HB7-Blocked Ricin + Excess HB7 Antibody</th>
<th>Maximal Cell Kill by HB7-Blocked Ricin</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS-Sultan</td>
<td>3.0 x 10^-10</td>
<td>6.0 x 10^-11</td>
<td>&gt;1.0 x 10^-3</td>
<td>&gt;3.75 x 10^-10 (SF = 1)</td>
<td>&gt;6 logs*</td>
</tr>
<tr>
<td>Namalwa</td>
<td>4.5 x 10^-12</td>
<td>7.0 x 10^-12</td>
<td>2.5 x 10^-9</td>
<td>ND</td>
<td>=5 logs†</td>
</tr>
<tr>
<td>RPMI 8226</td>
<td>3.0 x 10^-12</td>
<td>7.0 x 10^-12</td>
<td>ND</td>
<td>&gt;1 x 10^-10 (SF = 1)</td>
<td>ND</td>
</tr>
<tr>
<td>U266B1</td>
<td>2.0 x 10^-11</td>
<td>&gt;2.0 x 10^-8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: SF, surviving fraction of cells; ND, not done.
* Cells were exposed to 8 x 10^-10 mol/L HB7-blocked ricin.
† Cells were exposed to 1.5 x 10^-10 mol/L HB7-blocked ricin. Cells were incubated with a toxin for 24 hours at 37°C in a humidified atmosphere containing 5% CO2. Cells were then washed, placed in fresh medium, and the surviving fractions of cells were determined as outlined in Materials and Methods. The survival of U266B1 cells exposed to 0.25 nmol/L, 6.5 nmol/L, 1 nmol/L, or 2 nmol/L of HB7-blocked ricin under these conditions was 100%. IC50 values were interpolated from the cytotoxicity curves as an index of the ability of the toxin to kill cells. IC50 is the concentration of a toxin leaving a surviving fraction of 0.5. In a one-hit/killing model, a surviving fraction of 0.37 (1/e, where e is the natural logarithm base) is left when the number of lethal hits received by a population of cells is equal to the number of cells in that population.
targeted against antigens expressed by CFU-GM, BFU-E, and CFU-GEMM cells.

Inability of HB7 antibody to induce activation of normal human PB lymphocytes. Normal human resting peripheral T cells do not express appreciable amounts of CD38 antigen on their surface. However, it has been reported that A10, an anti-CD38 antibody, activates human T cells. Such an activity would severely limit the clinical utility of HB7 antibody. Therefore, we examined whether HB7 antibody could induce activation of normal human peripheral T cells (Table 4). Resting normal PB lymphocytes were cultured with HB7 antibody under various conditions that had been previously reported as sufficient for activating T cells with A10 or other antibodies capable of activating T cells. Cells were cultured either in growth medium that contained HB7 antibody, or in plastic plates that had HB7 immobilized to their surfaces. As a positive control, cells were incubated with either a mitogenic combination of anti-CD2 antibodies, anti-T11, and anti-T11, or in plates that had an anti-CD3 antibody immobilized to their surfaces. As a negative control, cells were cultured in the absence of antibodies. The tritiated thymidine incorporation by the cells was then measured as an index of their activation. These experiments showed (Table 4) that, unlike the A10 antibody, HB7 does not induce activation of PB lymphocytes. However, in a manner similar to that reported for the A10 antibody, HB7 antibody enhances the mitogenic potential of anti-T11 antibody.

Cytotoxicity of HB7-blocked ricin for nonactivated human peripheral T cells. We also tested whether HB7-blocked ricin was toxic for nonactivated normal human T cells (Table 5). HB7-blocked ricin was toxic for the cells at 10^{-7} mol/L, the highest concentration tested, consistent with the fact that nonactivated peripheral T cells do not express CD38 antigen. As a positive control, nonactivated T cells were exposed to anti-CD6-blocked ricin. Human T cells express this antigen on their surface and as expected, anti-CD6-blocked ricin was toxic for the cells.

Effect of HB7-blocked ricin on multiple myeloma cells isolated from patients. In our attempt to test the efficacy of HB7-blocked ricin, we faced two problems. First, multiple myeloma clonogenic cells might differ from the main population of patients’ multiple myeloma cells in their CD38.
expression and, therefore, in their sensitivity towards the cytotoxic effect of HB7-blocked ricin. In addition, it is difficult to reproducibly maintain multiple myeloma cells in culture for more than 2 days. Therefore, we assayed for inhibition of protein synthesis after a short-term exposure (2 days) of freshly isolated patients’ cells to immunotoxin as an index of the cytotoxic potency of HB7-blocked ricin on these cells. Multiple myeloma cells from five patients have been tested (range 20% to 70% myeloma cells) and compared with samples of normal BM from two healthy volunteers (Fig 5). CD38-expressing cell lines HS-Sultan and RPMI 8226 were used as positive controls in these experiments. Protein synthesis in cells from four patients was inhibited at low doses (between 10 and 50 pmol/L) of HB7-blocked ricin (panel A), indicating that these cells were 100- to 500-fold more sensitive than the two samples of normal BM (panel C). Cells from one patient (panel B) were more resistant than those from the other four patients towards the effects of the immunotoxin, but still appeared to be more sensitive than the normal BM. The reason for this resistance is unclear. As expected (Table 2), HS-Sultan and RPMI 8226 cells were sensitive toward HB7-blocked ricin in this test (panel D).

To determine whether the inhibition of protein synthesis induced in cells by HB7-blocked ricin was mediated via CD3 interaction, cells were exposed to a conjugate of blocked ricin with an antibladder carcinoma murine MoAb BC-4E8 that does not bind to cells of hematopoietic lin- eages. As expected, plasma cells of the myeloma patients and the myeloma cell lines were much less sensitive to this immunotoxin (open symbols; Fig 3, A, B, and D) compared with HB7-blocked ricin (corresponding closed symbols). Control experiments confirmed that BC-4E8–blocked ricin was highly potent in killing the antigen-expressing SCaBER cell line (N. Kedersha, unpublished results, March 1991).

**DISCUSSION**

We describe an immunotoxin, HB7-blocked ricin, that is selectively highly cytotoxic towards cells expressing the CD38 antigen. At concentrations that show kill of several logs of CD38+ tumor (myeloma) cells, little toxicity to hematopoietic progenitors was observed. The highly potent killing of antigen-bearing tumor cells coupled with the lack of significant effects on PB mononuclear or hematopoietic progenitor cells suggests that HB7-blocked ricin may be useful for the therapy of antigen-bearing malignancies, such as multiple myeloma.

We chose the CD38 antigen as a target because it is universally and strongly expressed on all cells among all myeloma clones obtained from patients. Most antigens other than CD38 are restricted in their expression on myeloma cells. Because it remains unclear which cells within the myeloma clones are capable of self-renewal, we chose to target the entire malignant cell population. When tested on Namalwa cells, HB7-blocked ricin was comparable in potency with an anti-CD19–directed immunotoxin, anti-B4–blocked ricin, both in (1) the IC50 values (7 × 10−12 V 1 × 10−11 mol/L), which reflect the capacity of the immunotoxins to kill the majority of cells in the cell population; and (2) log kill (±5 logs for both immunotoxins), which is a measure of the capacity of the immunotoxins to kill rare resistant variant cells. In our studies, the binding pattern of HB7 antibody to cell lines of B, T and myeloid/monocytic origin was similar to that described for other anti-CD38 antibodies. In particular, it was strongly expressed on myeloma, B- and T-cell acute lymphoblastic leukemias, as well as on Burkitt’s and

| Table 4. Ability of Antibodies to Activate Resting Human PB Lymphocytes |
|-----------------------------|----------------|
| Antibody Added to PB Lymphocytes | [3H]d incorporation, cpn x 10−3 Mean ± SEM (n = 3) |
| None (medium) | 0.3 ± 0.1 |
| HB7 (60 nmol/L) in medium | 0.35 ± 0.04 |
| HB7 (0.3 μmol/L) in medium | 0.8 ± 0.2 |
| anti-T11 (0.12 μmol/L) | 0.5 ± 0.2 |
| anti-T11 (0.12 μmol/L) | 0.50 ± 0.04 |
| anti-T11 (0.12 μmol/L) + anti-T11 | 3.10 ± 0.10 |
| HB7 (60 nmol/L) + anti-T11 (0.12 μmol/L) in medium | 0.53 ± 0.09 |
| HB7 (60 nmol/L) + anti-T11 (0.12 μmol/L) in medium | 1.6 ± 0.1 |
| HB7 (0.3 μmol/L) + anti-T11 (0.12 μmol/L) in medium | 1.3 ± 0.3 |
| HB7 (0.3 μmol/L) + anti-T11 (0.12 μmol/L) in medium | 2.1 ± 0.3 |
| HB7 immobilized | 0.2 ± 0.1 |
| anti-CD3 adhered | 88 ± 4 |

**Table 5. Cytotoxicity of HB7-Blocked Ricin for Normal Resting Human Peripheral T Cells**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Plating Efficiency*</th>
<th>Surviving Fraction of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium only</td>
<td>0.29</td>
<td>1.00</td>
</tr>
<tr>
<td>Anti-CD6–blocked ricin (1 nmol/L), a positive control</td>
<td>0.028</td>
<td>0.994</td>
</tr>
<tr>
<td>HB7-blocked ricin (0.1 nmol/L)</td>
<td>0.37</td>
<td>1.26</td>
</tr>
<tr>
<td>HB7-blocked ricin (1 nmol/L)</td>
<td>0.26</td>
<td>0.89</td>
</tr>
<tr>
<td>HB7-blocked ricin (1 nmol/L) + HB7 (100 nmol/L)</td>
<td>0.39</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Freshly isolated normal resting PB lymphocytes were incubated in growth medium with immunotoxin at 37°C for 24 hours, and then washed.

* Plating efficiency is a fraction of plated cells that form colonies. Plating efficiencies and the surviving fractions of cells were established in a clonogenic assay as described in Materials and Methods.
some non-Hodgkin’s lymphomas. Because this antigen is found on malignant cells in virtually all patients suffering from multiple myeloma, such an immunotoxin may be an efficacious agent for treatment of this disease. Nonetheless, because the phenotype of the clonogenic cell in myeloma is undefined and theoretically this cell could lack surface CD38 expression, the therapeutic value of HB7-blocked ricin remains to be defined in clinical studies.

Normal resting peripheral T cells do not express appreciable amounts of CD38 antigen, suggesting that HB7-blocked ricin should not cause any adverse effects on these cells. Indeed, HB7-blocked ricin does not kill resting T cells, which lack CD38 expression. The A10 MoAb, also directed at the CD38 antigen, can augment proliferation of normal human T cells and lines expressing CD38 independently of accessory cells or IL-2. Therefore, we similarly examined HB7 MoAb and our studies showed that HB7 antibody does not induce T-cell activation; our previous studies have shown that HB7 does not cause proliferation of myeloma cell lines. However, HB7 antibody has a weak comitogenic activity toward T cells when applied together with anti-Ti11. The mechanism of this phenomenon and its in vivo significance is not clear.

HB7-blocked ricin showed limited toxicity for normal hematopoietic progenitors of the BM. Experiments with admixtures of a tumor cell line with normal BMMCs showed more than 2 logs of tumor cell kill under conditions that were not very toxic to the marrow. This toxicity appears to be nonspecific because it is in the same range as the one observed for anti-My9-blocked ricin (anti-My9 is an anti-CD33 antibody), an immunotoxin that binds to an antigen not expressed on BM clonogenic cells. This would confirm previous reports that hematopoietic stem cells do not express CD38 antigen. Indeed, hematopoietic colonies from CD34+CD38+ progenitor cells have been generated that are consistent with the view that depletion of CD38+ cells may not deplete or lineage committed CD34+CD38+ progenitor cells. Thus, HB7-blocked ricin appears to be safe as a BM purging agent.

In addition to multiple myeloma, anti-CD38—directed blocked-ricin conjugates may also have potential for a number of other therapeutic applications that involve eradication of CD38+ cells such as lymphomas and leukemias, as well as in vivo (systemic) and ex vivo suppression of autoimmune disease by killing activated T cells.

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