Evaluation of CD38 as Target for Immunotherapy in Multiple Myeloma

To the Editor:

We have read with interest the report of Goldmacher et al. They describe the construction of an anti-CD38 immunotoxin (IT) by conjugating CD38 monoclonal antibody (MoAb) HB7 to blocked ricin. The resulting IT shows potent killing of plasma cell lines, whereas only low toxicity is observed for hematopoietic precursor cells (HPC). Therefore, they conclude that this HB7-blocked ricin may have clinical utility for ex vivo and in vivo therapy of multiple myeloma (MM). We agree with the notice that CD38 is strongly expressed on malignant (and normal) plasma cells, but differ in opinion about the side effects of an anti-CD38 IT. These side effects relate to reactivity with HPC as well as with nonlymphoid tissue.

Our research group is also engaged in the development of an MoAb based therapy for MM. We performed a selection procedure with a panel of 12 different plasma cell reactive MoAbs, including four CD38 MoAbs: ID5 (IgM), 6B10 (IgM), and 7A6 (IgG2a) from R. van Lier (Amsterdam, The Netherlands) and also HB7 (IgG1) from M. Cooper (Birmingham, AL).

For ex vivo use, we used as major criterium (besides reactivity with malignant cells) the absence of reactivity with HPC to avoid interference with blood cell repopulation of the treated bone marrow. Goldmacher et al correctly mention that the pluripotent HPC are crucial for a long-term (sustained) marrow recovery. These cells are functionally identified by primitive blast colony formation and are described as CD34⁺CD38⁻. However, committed HPC, which are usually tested for in colony assays, are thought to be essential for a rapid repopulation and a shortening of the immunocompromised period of the patient. Goldmacher et al report toxicity of their HB7-blocked ricin to HPC in these assays, but only at concentrations much higher than the effective concentration on plasma cells. They state that the toxicity found is caused by aspecific toxicity of the ricin moiety, because it is comparable to the toxicity of an anti-CD33—blocked ricin. Firstly, it is not clear to us what the investigators mean by "aspecific toxicity," because CD33 is expressed by granulocyte-macrophage colony-forming unit (GM-CFU) cells and these cells may very well be specifically eliminated by the IT. With respect to the effect of CD38 on colony assays, we performed burst-forming unit-erythroid (BFU-E) and CFU-GM assays with bone marrow treated with the complement fixing (which excludes HB7) CD38 MoAbs and rabbit complement (Table 1).

Depletion of CD38⁺ cells resulted in a complete eradication of GM-CFU cells and a practically complete one of BFU-E cells. No effect was seen with the MoAbs alone (data not shown). Whereas the quantitative difference between our results and those of Goldmacher et al might relate to a different density of CD38 or a possible different IT-induced internalization of CD38 on HPC than on plasma cells, it certainly enforces the caution that the toxicity they saw with their anti-CD38 IT is a specific one indeed.

The importance of a bone marrow purging protocol for MM may be restricted by the recent progress in the application of peripheral HPCpheresis and transplantation. Therefore, Goldmacher et al rightly envisage as well an eventual in vivo therapy. An additional criterium to reactivity with hematopoietic precursors for such in vivo use is the absence of crossreactivity with other tissues, an item not discussed or tested by Goldmacher et al. Indeed, sufficient data are lacking in the literature so far. We have tested the crossreactivity of CD38 MoAb with different tissues using indirect three-step immunoperoxidase staining. Tissues involved included spleen, stomach, duodenum, esophagus, tonsil, thyroid gland, liver, lung, kidney, heart, muscle, skin, thymus, brain, lymph node, skin immunocytoema, and nasopharynx plasmacytoma. Plasma cells at various locations

<table>
<thead>
<tr>
<th>Table 1. Hematopoietic Precursor Assays With CD38-Depleted Fractions</th>
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<tbody>
<tr>
<td><strong>No. of Colonies per 2 x 10⁶ Cells</strong></td>
</tr>
<tr>
<td>BFU-E</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Complement</td>
</tr>
<tr>
<td>1D5 + complement</td>
</tr>
<tr>
<td>6B10 + complement</td>
</tr>
<tr>
<td>7A6 + complement</td>
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Data from two donors are presented.
Table 2. Nonlymphoid Reactivity of Four CD38 MoAbs

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>1D5</th>
<th>9B10</th>
<th>7A6</th>
<th>HB7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Some blood vessels in duodenum</td>
<td>Duodenum epithelium</td>
<td>Duodenum epithelium and some vessels</td>
<td>Macrophages along sinusoids of liver</td>
<td>Epithelium in alveoli and bronchi(o)l of lung</td>
</tr>
<tr>
<td>Macrophages along sinusoids of liver</td>
<td>Lamina basalis and some cells in epidermis of skin</td>
<td>Liver vessels and macrophages along sinusoids</td>
<td>Epithelium and vessels of skin</td>
<td>Capillaries of heart</td>
</tr>
<tr>
<td>Epithelium in bronchi(o)l of lung</td>
<td>Duodenum epithelium</td>
<td>Duodenum epithelium</td>
<td>Macrophages along sinusoids of liver</td>
<td>Epithelium and vessels of skin</td>
</tr>
<tr>
<td></td>
<td>Some vessels of tonsil</td>
<td>Some vessels of lymph gland</td>
<td>Epithelium and vessels of skin</td>
<td>Capillaries of heart</td>
</tr>
<tr>
<td></td>
<td>Thymic basophil</td>
<td>Thymic basophil</td>
<td>Capillaries of heart</td>
<td>Vessels of lymph gland</td>
</tr>
<tr>
<td></td>
<td>Some vessels of lymph gland</td>
<td>Epithelium and vessels of skin</td>
<td>Vessels of lymph gland</td>
<td>Epithelium and vessels of skin</td>
</tr>
</tbody>
</table>

as well as germinal centers in lymphoid organs were positive. In addition, thymocytes in cortex and medulla were stained. Apart from these lymphoid components, reactivity with other tissues and cells was present, as summarized in Table 2. There seem to be subtle differences between the four CD38 MoAbs; 7A6 and HB7 showed similar staining patterns with more intense and extensive reactivity than 6B10 and 1D5.

The complication of tissue destruction due to the unwanted binding to tissue components may not be necessarily true to its full extent because the immunohistochemistry of tissue sections ex vivo may differ from accessibility of cells in organs in vivo; this accessibility in vivo is indeed larger for endothelium than for epithelium. Expression of CD38 by basophils, as mentioned by the investigators and also found by us in tissue sections, is an important drawback. Destruction of these cells by anti-CD38 IT may result in anaphylactic shock caused by the release of vasoactive agents.

From our results we reached the conclusion that CD38 MoAbs are principally not the right candidates for immunotherapy either ex vivo or in vivo in patients with MM.

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REFERENCES


Response

Vooijs et al suggest that anti-CD38 monoclonal antibody HB7 conjugated to blocked ricin may not be therapeutically useful for treatment of myeloma either ex vivo or in vivo because of potential side effects. The first potential toxicity site is on hematopoietic progenitor cells (HPC). It is impossible at present to test for HPC toxicity, given the lack of assays to identify the pluripotent hematopoietic stem cell. However, as Vooijs et al and we noted, CD38 is not expressed on primitive hematopoietic precursors, which are CD34+CD38—. These presently can be measured by long-term culture-initiating cell (LTCIC) and pre-colony-forming unit—granulocyte-macrophage (CFU-GM) or delta assays. Their phenotype is CD34+CD38—DR+Thyl+, lineage+,4 rhodamine dull. Neither Vooijs et al nor we performed LTCIC or delta assays; however, the lack of CD38 expression on these cells likely predicts minimal effects for CD38-targeted cytotoxic agents.

Committed progenitor cells, which are CD38+, were analyzed in our studies and those of Vooijs et al. The anti-CD38 HB7-blocked ricin immunotoxin used in our study was only moderately toxic for CFU-GM, burst-forming unit-erythroid (BFU-E), and colony-forming unit—granulocyte, erythroid, macrophage, monocyte (CFU-GEMM) at concentrations of 10−16 to 10−11 mol/L, whereas it was effective in eradicating CD38+ tumor cells. Anti-CD38 monoclonal antibodies and complement used by Vooijs et al completely abrogated growth of BFU-E and CFU-GM. We agree with Vooijs et al that this difference in toxicities of the immunotoxin and anti-CD38 and complement treatment could be caused by the different densities of CD38 on the tumor cells and the progenitor cells. The toxicity of HB7-blocked ricin towards CFU-GM, BFU-E, and CFU-GEMM cells observed in our study was similar to that of anti-CD33-blocked ricin reported previously, probably reflecting the expression of both these antigens on committed progenitors used in clonogenic assays, ie, their toxicity for these progenitors is likely to be specific, as rightly pointed out by Vooijs et al. However, the study of anti-CD33—blocked ricin showed that the immunotoxin was less toxic than anti-CD33 + complement treatment for the CD33-expressing bone marrow progenitor cells. Furthermore, anti-CD33—blocked ricin has now been used ex vivo to purge autologous marrow of patients with acute myeloid leukemia before transplantation, and complete engraftment was observed. Both anti-CD38—blocked ricin and anti-CD33—blocked ricin were more cytotoxic for tumor cells than for progenitor cells, and we believe both agents could be efficacious for ex vivo use. It is interesting to note that Stevenson et al have reported that a chimeric antibody consisting of the Fab from a mouse anti-CD38 monoclonal antibody bound to an Fc molecule derived from human IgG1 was effective in mediating antibody-dependent cellular cytotoxicity with blood mononuclear effector cells, while not altering the growth of BFU-E or CFU-GM cells.

With regard to the use of anti-CD38—blocked ricin for in vivo therapy, it is clearly important to carefully evaluate the distribution of CD38 expression in tissues other than in the hematopoietic compartment. The new interesting data reported by Vooijs et al in their letter suggest that CD38 is expressed on a variety of tissues. How-
ever, immunohistochemical analysis of tissue sections is often inconclusive because the technique does not clearly discriminate between antigen expression inside and outside a cell, nor is it easy on the basis of such data to evaluate the relative densities of an antigen on normal and tumor cells. Furthermore, Stevenson et al (personal communication, January 1995) have now treated a single patient with multiple myeloma and six patients with B-cell lymphoma using anti-CD38 chimeric antibody and have observed no hematologic or other organ toxicity. These data suggest that anti-CD38-blocked ricin may also be useful in vivo, but we acknowledge that thorough preclinical evaluation of cross-reactivity will be necessary before proceeding with therapeutic trials.

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Evaluation of CD38 as target for immunotherapy in multiple myeloma [letter; comment]

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