Ligation of Cell Surface CD38 Protein with Agonistic Monoclonal Antibody Induces a Cell Growth Signal in Myeloid Leukemia Cells

Marina Konopleva, Zeev Estrov, Shourong Zhao, Michael Andreeff and Kapil Mehta

*J Immunol* 1998; 161:4702-4708; [http://www.jimmunol.org/content/161/9/4702](http://www.jimmunol.org/content/161/9/4702)

---

**References**

This article cites 43 articles, 22 of which you can access for free at: [http://www.jimmunol.org/content/161/9/4702.full#ref-list-1](http://www.jimmunol.org/content/161/9/4702.full#ref-list-1)

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions**

Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
Ligation of Cell Surface CD38 Protein with Agonistic Monoclonal Antibody Induces a Cell Growth Signal in Myeloid Leukemia Cells

Marina Konopleva,* Zeev Estrov,† Shourong Zhao,* Michael Andreeff,* and Kapil Mehta†‡

CD38 is expressed during early stages of differentiation in normal and leukemic myeloid cells. Recently, CD38 has been shown to participate in intracellular signal transduction pathways following its ligation with CD38-specific mAbs. In this study we report that ligation of CD38 by one such agonistic mAb (IB4) induced proliferation of cultured leukemic cells in vitro. In HL-60, KG-1A, NB4, and OCI-AML-3 myeloid leukemia cell lines, IB4 mAb induced an increase in the proliferating cell fraction as determined by cell number, clonogenic assay, and flow cytometric analysis. The presence of Ab caused a dose-dependent increase in the number of CFU and an increase in cell divisions. HL-60-Dox cells (a HL-60-doxorubicin-resistant cell line), which have no detectable CD38 expression, failed to respond to IB4 mAb. The effect of CD38 ligation on cell growth was also evaluated in freshly isolated leukemic cells from patients with acute myelogenous leukemia (AML). A significant increase in the proliferating cell fraction (S+G2M) was observed in 50% of the patients incubated with IB4 mAb. In five of the six AML patients, anti-CD38 mAb stimulated the proliferation of AML colony-forming cells. These results suggest that ligation of CD38 can induce the proliferation of leukemic cells and may play a role in the propagation of leukemic cell clones in certain cohorts of AML patients. The Journal of Immunology, 1998, 161: 4702–4708.

H

uman CD38 is a nonlineage-restricted type II transmembrane glycoprotein that is predominantly expressed by immature and activated cells of different lineages (1–4). A high sequence homology was recently reported between mouse and human CD38 and Aplysia californica ADP ribosyl-cyclase (5).

Like the Aplysia cyclase enzyme, CD38 can catalyze the synthesis of cyclic ADP-ribose (cADPR)3 (3) from NAD+ and can further hydrolyze cADPR to ADP-ribose (6–8). The cADPR has potent Ca2+-mobilizing activity and is a potential endogenous regulator of calcium-dependent calcium release (9). The ability of cADPR to release calcium from intracellular pools is thought to be part of certain CD38-mediated signaling pathways that result in cell growth, apoptosis, and differentiation (3). The involvement of CD38 as a receptor for an as yet unidentified ligand has also been suggested by a wide variety of physiologic events in lymphocytes that are elicited following its ligation with an agonistic mAb (10–13). Recently, CD31 belonging to the Ig superfamily of cell surface proteins was described to function as one such ligand for CD38. Thus, interaction between CD38 and CD31 could elicit cytoplasmic calcium fluxes and induction of certain cytokine transcripts, identical with those mediated by anti-CD38 agonistic mAb (14).

Within the myeloid lineage, CD38 is mainly expressed by precursor cells, and only weak expression has been observed on mature myeloid cells (15). Its expression has also been reported on the malignant counterparts of these hemopoietic cells, and CD38 may be of prognostic relevance in acute myelogenous leukemia (AML) (16). Recently, it was reported that all-trans-retinoic acid (ATRA) is a highly potent and specific inducer of CD38 expression in human myeloid leukemia cells (17–20). ATRA-induced expression of the CD38 Ag is mediated via activation of the retinoic acid receptor-α type of nuclear receptor (19, 20). However, not much information is available on the possible function of CD38 in myeloid leukemia cells.

The CD38 Ag is one of the surface proteins whose receptor activity has been demonstrated by means of specific agonistic mAbs (10, 13). It is well known that the binding of a mAb to a receptor molecule could be followed either by a functional block or by activation effects. The activation effects ensue if recognition of different epitopes by the mAb mimics the events triggered by the natural ligand of the receptor. In hemopoietic cells, the ligation of cell surface CD38 with agonistic mAbs has been shown to trigger such diverse responses as cell growth, stimulation and prevention of apoptosis, and protein tyrosine phosphorylation in hemopoietic cells (10–13, 21–24). These observations suggest that CD38 could serve as a receptor and mediate signal transduction pathways leading to cell growth, apoptosis, and cytokine production. To elucidate the role of the CD38 protein in myeloid leukemia cells, we also used a CD38-specific mAb to mimic the effects of the putative natural ligand for this molecule. The results obtained suggest that ligation of the cell surface CD38 protein provides a potent cell growth signal in myeloid leukemia cells.

Materials and Methods

Abs and cell lines

The anti-CD38 mAbs, IB4 (IgG2a) and IB6 (IgG26), were provided Dr. Fabio Malavasi (Turin, Italy) (25). The isotypic control IgG2a κ was purchased from Sigma (St. Louis, MO). The HL-60 and KG-1 cell lines were

3 Abbreviations used in this paper: cADPR, cyclic ADP ribose; AML, acute myeloid leukemia; ATRA, all-trans-retinoic acid; HL-Dox, HL-60-doxorubicin-resistant cell line; PE, phycoerythrin.

Departments of *Hematology and †Bioimmunotherapy, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Received for publication January 20, 1998. Accepted for publication July 1, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked
in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work was supported by grants from the Texas Higher Education Coordinating Board (ATP000015-012), grants from the National Institutes of Health (CA55164, CA49639, and CA16672), and the Union Internationale Centre le Cancer fellowship program (to M.K.).

Address correspondence and reprint requests to Dr. Kapil Mehta, Department of Bioimmunotherapy, Box 60, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030. E-mail address: kmehtha@mdacc.tmc.edu

Copyright © 1998 by The American Association of Immunologists 0022-1767/98/$02.00
obtained from the American Type Culture Collection (Manassas, VA); the NB4 cell line was provided by Dr. M. Lanotte (26). The HL-60-doxorubicin-resistant cell line (HL-Dox) has been used as a control (27). The OCI/AML3 cell line, originally established from an AML patient (28), was provided by Dr. M. D. Minden (Ontario Cancer Institute, Toronto, Canada). All cell lines were maintained in RPMI 1640 medium containing 10% FCS, 1% -glutamine, and penicillin-streptomycin. Cell number was adjusted to 0.5 × 10^6 cells/ml and cells were cultured in 96-well plates at 37°C in 5% CO_2 for 48 h in 5% CO_2 with anti-CD38 (IB4 or IB6) mAb or with control IgG2a at a predetermined optimal dose (5 μg/ml). After 6, 8, 24, and 48 h of culture, the number of viable cells was determined using the trypan blue exclusion test.

**Subjects**
Bone marrow or peripheral blood cells for in vitro studies were obtained from newly diagnosed AML patients after obtaining their informed consent according to the institutional policy. The mononuclear cells were separated by Ficoll-Hypaque (Sigma) density gradient centrifugation. All samples tested contained >70% blasts. The clinical features are listed in Table I. Triplicate samples of 1.0 × 10^6 cells suspended in 200 μl of RPMI medium were cultured in the presence or the absence of IB4 (5 μg/ml) in 96-well flat-bottom microtiter well plates (Costar, Cambridge, MA) in a humidified atmosphere of 5% CO_2 in air at 37°C for 2 days and were used for different assays.

**Clonogenic assay**
The clonogenic assay was performed as previously described (29). Briefly, 2 × 10^3 OCI/AML3 cells were cultured in 0.8% methylcellulose (Fluka, Ronkonkoma, NY), 10% FBS, and Iscove’s modified Dulbecco’s medium. Anti-CD38 mAbs, IB4 or IB6, or isotypic control Ig (IgG2a) were added at the initiation of cultures at concentrations ranging from 1 to 7.5 μg/ml. Triplicate culture mixtures were placed in 35-mm petri dishes (Nunc, Roskilde, Denmark) and stained to confirm leukemic cellular composition. As previously described, the AML blast colonies using this assay contained only the blasts and no normal progenitors (32).

**Cell kinetics studies**
The cell cycle kinetics were determined after 48 h of treatment with IB4 mAb by staining cells with acridine orange for examination of the cellular DNA and RNA contents followed by flow cytometric analysis. This method enables discrimination of cells in G_0, G_1, S, and G_M phases and determines the mean RNA content per cell during each phase of the cell cycle (33). Briefly, aliquots (80 μl) of cells were mixed with 100 μl of solution containing 0.1% (v/v) Triton X-100, 0.05 N HCl, 0.15 M NaCl, and 8 μg/ml acridine orange (Polysciences, Warrington, PA). The cell fluorescence was measured within 5 min of staining. Samples were measured in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) using the 488 nm line of a 15 nm argon laser and filter settings for green (530 nm; DNA) and red (585 nm; RNA) fluorescence. Ten thousand events were stored in list mode for analysis. The percentage of cells in the sub-G_1 peak defined the proportion of apoptotic cells in the tested populations.

**Results**
The cells were resuspended in diluent C (Sigma) at 10^7 cells/ml. An equal volume of this cell suspension was then gently mixed with diluent C containing 4 μM PKH26-Gl (Sigma) and incubated for 5 min. The reaction was stopped with the addition of an equal volume of FCS. After 1 min an equal volume of RPMI 1640 containing 10% FCS was added, and the cells were washed three times in the same medium by centrifugation (400 × g, 25°C, 10 min). All staining procedures were conducted at room temperature. After staining, the cells were cultured with or without the anti-CD38 mAb, and fluorescence intensity was measured on days 1 through 5. The instrument was set up using the same laser power and gains as those being monitored with the fluorescent microspheres. The high voltage on the photomultiplier tube was adjusted until the intensity of standard microspheres was identical with that at time zero. The data were analyzed using the ModFit computer program (Sigma). The method allows determination of the number of divisions that individual cell undergoes, as each daughter cell has half the PKH-26 fluorescence of the parent cells (34). The Cell Prolieration Model software we used for these studies calculates the proliferation index, a ratio of the total number of cells analyzed vs the calculated number of parental cells required for the observed cell number. The total number of cells is determined by the summation of the Gaussian areas in each generation (A_k). The original number of cells is calculated by the summation of the Gaussian areas divided by 2^k, where k equals the generation number.

**Immunophenotyping**
The PE-conjugated anti-CD38 mAb (Leu-17; Becton Dickinson, San Jose, CA) was used at a 1/10 dilution. The staining procedure was performed as described previously (19). Positivity was defined by comparing the fluorescence of anti-CD38-labeled cells with that of PE-labeled IgG1 controls. The percentage of cells considered positive was calculated by subtracting the percentage of cells with a fluorescence intensity greater than the set marker using the isotype control (background) from the percentage of cells with a fluorescence intensity greater than the same marker using the specific Ab.

**Statistics**
The results are expressed as the mean ± SEM. Levels of significance were evaluated by two-tailed paired Student’s t test, and p < 0.05 was considered significant.

**Table I. Clinical data for patients with AML**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Cytogenetic Abnormality</th>
<th>FAB Category</th>
<th>WBC (&lt;10^9/L)</th>
<th>% Blasts in BM (Before DS)</th>
<th>% Blasts in PB (Before DS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>F</td>
<td>Diploid</td>
<td>M3</td>
<td>40.0</td>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>M</td>
<td>+13</td>
<td>M0</td>
<td>192.0</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>M</td>
<td>inv 16</td>
<td>Relapse</td>
<td>61.6</td>
<td>87</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>M</td>
<td>−7, inv 16</td>
<td>M4E0</td>
<td>200.0</td>
<td>76</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>F</td>
<td>−5, (t;16;17)</td>
<td>M0, relapse</td>
<td>1.4</td>
<td>78</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
<td>M</td>
<td>+11</td>
<td>M1</td>
<td>226.0</td>
<td>85</td>
<td>98</td>
</tr>
<tr>
<td>7</td>
<td>78</td>
<td>M</td>
<td>−5, −7</td>
<td>M2</td>
<td>15.2</td>
<td>77.4</td>
<td>63</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>F</td>
<td>Diploid</td>
<td>M0, relapse</td>
<td>7.4</td>
<td>89</td>
<td>75</td>
</tr>
</tbody>
</table>

* FAB, French-American-British; WBC, white blood cells; BM, bone marrow; PB, peripheral blood; inv 16, inversion 16; DS, density separation.
anti-CD38 mAb or control IgG2a. Treatment with IB4 mAb resulted in a significant increase in cell number in both cell lines (Fig. 1). Interestingly, treatment with another anti-CD38 mAb (IB6) under similar conditions caused no increase in cell numbers compared with that in control medium or medium containing isotypic Ab IgG2a. Similarly, CD38+ HL-60-doxorubicin-resistant cells incubated with IB4 mAb did not respond with increased cell growth. Cell cycle analysis of HL-60 and NB4 cells using acridine orange staining and flow cytometry further confirmed the ability of IB4 mAb to induce cell growth signal as revealed by an increase in the proliferative cell fraction (S+G2M fraction; Table II).

**IB4-induced increase in cell divisions**

Using fluorescent membrane dye, PKH26-GL, as a probe, it was possible to follow the cell division history of a cell in vitro. Following each cell division, the fluorescence intensity of each daughter cell is half that of the parental cell. As a result, the inverse fluorescence intensity progressively decreases as function of time and correlates with an increase in cell counts. After staining with PKH26, the cells were grown in the presence or the absence of anti-CD38 Ab and on different days were analyzed for their fluorescence intensity. The results shown in Table III demonstrate that treatment of the two CD38+ cell lines (NB4 and HL-60) with IB4 mAb induced a pronounced increase in the proliferative response compared with that of untreated control cells or IgG2a-treated cells. However, incubation with nonagonistic anti-CD38 mAb (IB6) under similar conditions failed to cause any increase in cell proliferation (data not shown). The ModFit computer program was used to deconvolute the raw histogram data into peaks that represent the different cell generations. It calculates the percentages of cells in different populations objectively without their interference. Histogram deconvolution data showed a significant \((p < 0.01)\) shift toward the latest cell generation in IB4-mAb-treated NB4 cells, which was most prominent on day 2 (Fig. 2). In CD38+HL-60-Dox cells no difference in cell kinetics was found in two independent experiments (data not shown).

To determine a correlation between the anti-CD38-induced proliferative response and the cell surface expression of CD38 Ag, we determined the levels of CD38 expression on various cell types by flow cytometry. The results shown in Table IV suggested that the mean fluorescence intensity was highest in the OCI/AML3 and NB4 cell lines. HL-60-Dox cells had no detectable expression of CD38 Ag, corresponding with their inability to respond to anti-CD38 Ab treatment.

**Anti-CD38 mAb stimulates colony formation and AML colony-forming cell proliferation**

To determine the effect of CD38 ligation on myeloid progenitor cells, we studied the colony-forming ability of the myeloid cell line OCI/AML3 in response to anti-CD38 mAb (IB4). The results demonstrated that the IB4 mAb significantly \((p < 0.001)\) improved the colony-forming ability of OCI/AML3 cells in a dose-dependent manner (Fig. 3A). A >80% increase in CFU was observed in Ab-treated cultures compared with that in untreated cultures (Fig. 3A). Interestingly, another anti-CD38 mAb (IB6) was completely inactive in stimulating the colony growth of OCI/AML3 cells even though the two mAbs reacted equally well with CD38 Ag as revealed by immunoblotting (Fig. 3B). Similarly, inclusion of isotypic Ig (IgG2a) in cultures even at the highest concentration (7.5 \(\mu\)g/ml) failed to promote the growth of CFUs (Fig. 3A).

Next, we examined the effect of anti-CD38 Ab on the proliferation of AML progenitor cells. For this purpose, we used fresh bone marrow cells from six patients newly diagnosed with AML who had a blast counts of >70%. The results revealed that in five of the six patients, IB4 mAb treatment caused a significant \((p = 0.0015)\) increase in the proliferation of AML colony-forming cells (Fig. 4). In contrast, anti-CD38 IB6 mAb did not affect the colony-forming ability of AML blasts in any of the samples tested. Neither IB4 nor IB6 anti-CD38 mAb induced any statistically significant \((p > 0.12)\) effect on the colony proliferation of granulocyte-macrophage CFU or erythroid burst-forming units when two normal low density bone marrow cells were used as controls (data not shown).

---

**Table II. Effect of IB4 mAb on cell kinetics in HL-60 and NB4 cells**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Generation No.</th>
<th>Control</th>
<th>IgG2a</th>
<th>+ IB4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HL-60</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0h</td>
<td>41.2 ± 1.9</td>
<td>40.0 ± 1.2</td>
<td>54.3 ± 4.1*</td>
<td></td>
</tr>
<tr>
<td>24h</td>
<td>43.9 ± 2.9</td>
<td>45.3 ± 2.7</td>
<td>48.0 ± 1.0*</td>
<td></td>
</tr>
<tr>
<td><strong>NB4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0h</td>
<td>43.2 ± 1.6</td>
<td>45.2 ± 2.0</td>
<td>54.3 ± 4.1*</td>
<td></td>
</tr>
<tr>
<td>48h</td>
<td>45.9 ± 2.8</td>
<td>46.3 ± 2.5</td>
<td>54.3 ± 4.1*</td>
<td></td>
</tr>
</tbody>
</table>

*Cells were incubated in medium alone or in medium containing the control (IgG2a) or anti-CD38 Ab (IB4, 5.0 \(\mu\)g/ml) for 48 h. The at the end of incubation period, cells were analyzed for cell cycle (phase) distribution by flow cytometry after staining with acridine orange as described in Materials and Methods. Results are expressed as the percent of cells in the proliferative fraction (cells in the S+G2M phase). Values represent mean ± SEM of three independent experiments. \(^*p < 0.05\) (paired \(t\) test).
Anti-CD38 mAb-mediated changes in AML cell kinetics

The clinical data of patients used for studying the anti-CD38-induced alterations in cell kinetics are summarized in Table I. The cells were isolated from fresh samples from AML patients and cultured in triplicate with or without the IB4 mAb. After 48 h of incubation, cell kinetics were determined by means of cell count and flow cytometry using acridine orange staining for cellular DNA and RNA contents. Cell cycle analysis by flow cytometry showed a significant \((p < 0.01)\) increase in the number of proliferating cells (S + G2 + M phase) in four of the eight patient samples studied in response to IB4 mAb treatment (Fig. 5). Interestingly, the incubation of cells in the presence of IB6 nonagonistic mAb under these conditions, failed to alter the proliferative response of AML cells in all eight samples; a representative example is shown in Figure 6. Similarly, samples from two patients with myelodysplastic syndrome (refractory anemia) and two patients with acute lymphoblastic leukemia did not exhibit any proliferative response following treatment with the IB4 mAb (data not shown). No significant differences in the percentage of apoptotic cells was found in IB4-treated cultures compared with that in controls (13.1 ± 1.2 vs 14.9 ± 2.1; \(p > 0.1\)).

CD38 expression in the leukemic (gated on scatter) cell population was determined using PE-conjugated mAb (Leu-6). Leukemic cells from all patients had a level of CD38 expression ranging from 46 to 100%, but no correlation between the level of CD38 expression, as determined by mean fluorescence intensity, and the proliferative response was observed.

Discussion

Human CD38 is a cell surface Ag that belongs to a family of molecules called accessory proteins. In many systems these accessory molecules are able to replace or enhance the biochemical signals provided by the cell-specific receptors (35, 36). In other instances, they mediate homo- and heterotypic adhesion (37). Furthermore, some members of this family can catalyze precise enzymatic functions (38, 39). The first experimental evidence that CD38 might be involved in signal transduction pathways came from the studies published by Funaro et al. (1). These authors...
demonstrated that Ab-induced dimerization of CD38 on the surface of PBMC and T cell lines induces a potent signal for activation and proliferation. Subsequent experiments demonstrated that ligation of human CD38 with specific mAbs induces the transcription of several cytokines at levels similar to those obtained after ligation of the T cell receptor CD3 (12, 40). However, the signal induced by CD38 ligation was distinct from that induced by ligation of T and B lymphocyte-specific receptors.

AML cells though lose their ability to differentiate, but, similar to normal myeloid precursor cells, they continue to proliferate in the bone marrow. The precise molecular mechanisms that control the growth and differentiation events in these cells are not understood. Similar to their normal counterparts, AML cells express the cell surface CD38 Ag. The results presented in this paper provide evidence that cell surface expression of CD38 may contribute to the regulation of proliferative signals in AML cells. Ligation of cell surface CD38 protein with an agonistic mAb induced a proliferative signal in the myeloid leukemia cells. Both, the CD38+ myeloid leukemia cell lines and the freshly isolated AML cells responded to the proliferation-inducing effect of anti-CD38 mAb, as determined by cell growth, clonogenic assay, flow cytometric analysis of DNA/RNA, and cell division analysis using the membrane-bound PKH26 dye. A proliferative response to anti-CD38 mAb treatment was observed only in cell lines that expressed detectable CD38. However, not all CD38+ patient samples responded to the mAb, indicating that AML cells from these patients may have some downstream defect in the signal transduction pathway. Indeed, CD38 ligation has been shown to result in rapid tyrosine phosphorylation of a discrete set of cellular proteins (21–24). One of the major proteins that are tyrosine phosphorylated following CD38 ligation was identified as the c-cbl proto-oncogene product, p120 c-cbl (22). Anti-CD38-induced tyrosine phosphorylation of c-cbl could serve as a docking site for certain cytoplasmic kinases, such as the 85-kDa regulatory subunit of

![FIGURE 4. Anti-CD38 mAb-induced proliferation of clonogenic AML cells. T cell-depleted, nonadherent bone marrow cells from nine AML patients were cultured in duplicate in a semisolid medium containing recombinant human granulocyte-macrophage CSF and agonistic (IB4) or nonagonistic (IB6) anti-CD38 mAb. After 7 days, the plates were evaluated under a microscope for the number of AML blast colonies, and the average numbers of CFU were plotted against the Ab concentrations.](http://www.jimmunol.org/)

![FIGURE 5. Anti-CD38 Ab-induced proliferation of AML blasts. The cell kinetics of freshly isolated samples from 8 AML patients were analyzed after 48-h in vitro treatment with the IB4 mAb. Following the appropriate treatment, the cells were stained with acridine orange and analyzed on a FACScan flow cytometer using a 488-nm line of a 15 nm argon laser and filter settings for green (530 nm) and red (585 nm). Data were analyzed using the LYSIS II computer program (Becton Dickinson). The results are expressed as the percentage of proliferating cells (cells in S+G2+M phase). Values represent the mean of triplicate determinations. §, p < 0.01 vs unstimulated cells.](http://www.jimmunol.org/)

![FIGURE 6. DNA content frequency histogram of AML patient cells, untreated (control) or cultured in the presence of anti-CD38 mAb (IB6 or IB4). The cells were stained and measured by flow cytometry as described in Materials and Methods. IB4 treatment was followed by an increase in cells in S+G2+M phase of the cell cycle.](http://www.jimmunol.org/)
phosphatidylinositol 3-kinase (23, 24), and may provide a signal for cell growth.

Another interesting observation was the inability of the IB6 mAb to transduce cell growth signal despite its ability to bind the cell surface CD38 as effectively as does the agonistic anti-CD38 mAb (IB4). These observations suggest that engagement of specific epitopes on the surface CD38 protein by agonistic mAbs is critical for effective signal transduction. Using several deletion mutants of the CD38 protein, Hoshino et al. (41) recently demonstrated that epitopes recognized by agonistic anti-CD38 mAbs, including the IB4 mAb, are localized at the carboxy-terminal sequence of 273 to 285. It is therefore likely that nonagonistic mAbs, such as IB6, recognize an epitope on the CD38 protein that is not localized in the C273–285 region and thus are unable to transmit a functional signal for cell growth.

An important question that these studies raise is whether agonistic mAbs mimic and represent the function of an as yet unidentified factor in vivo. Certain molecules present in bone marrow stromal or on endothelial cells have been shown to interact and effectively transduce a signal in CD38+ cells. For example, a 120-kDa protein that was recently identified as CD31 has been suggested to serve as a ligand for human CD38 in endothelial cells (14, 42). Similarly, a glycosylphosphatidylinositol-anchored protein, BST-1, which facilitates growth in pre-B cells, was cloned from a bone marrow stromal cell line (43). The amino acid sequence of BST-1 exhibited 33% identity with CD38. It is likely that in vivo engagement of CD38 on myeloid leukemia cells by such molecules (either on stromal cells or coexpressed on myeloid leukemia cells) may provide a signal for cell growth. Alternatively, ligation of the CD38 protein may prolong the life of the cells in vivo by preventing them from undergoing apoptosis and thus may result in the propagation of leukemic cells. Our preliminary data demonstrate that cocultures of CD38+ myeloid leukemia cells with CD31-transfected NIH-3T3 cells, induces a potent signal for cell growth, whereas the CD38+ leukemia cell lines IC2 and TEK do not respond (S. Umar and K. Mehta, unpublished observations). It is equally possible that CD38-mediated induction of such cytokines may contribute to the propagation of leukemia clones (44).

In conclusion, the results presented in this paper suggest that CD38-mediated signaling pathways may play a role in the propagation of leukemic clones in patients with myeloid leukemia. This effect of CD38 ligation may be mediated directly by activation of the cell growth signaling pathways or indirectly as a result of cytokine production.

References


