Expression of CD21 Antigen on Myeloma Cells and its Involvement in Their Adhesion to Bone Marrow Stromal Cells

By Naihui Huang, Michio M. Kawano, Maged S. Mahmoud, Keiichiro Mihara, Takako Tsujimoto, Otsura Niwa, and Atushi Kuramoto

The mature myeloma cells express very late antigen 5 (VLA-5) and MPC-1 antigens on their surface and adhere to bone marrow (BM) stromal cells more tightly than the VLA-5"MPC-1" immature myeloma cells in vitro. The VLA-5 and MPC-1 antigens possibly function as two of the molecules responsible for interaction of mature myeloma cells with BM stromal cells. However, the immature myeloma cells do interact with BM stromal cells, and it is unclear which adhesion molecules mediate their interaction. In this study, we found that both immature and mature myeloma cells expressed CD21, an adhesion molecule known to bind to CD23. CD21 was also detected on normal plasma cells. To evaluate the role of CD21 expression on myeloma cells, two myeloma cell lines, NOP-2 (VLA-5"MPC-1") and KMS-5 (VLA-5"MPC-1"), were used as representatives of immature and mature myeloma cell types, respectively, and an adhesion assay was performed between the myeloma cell lines and BM stromal cells. Antibody-blocking results showed that adhesion of the mature type KMS-5 to KM102, a human BM-derived stromal cell line, or to short-term cultured BM primary stromal cells was inhibited by monoclonal antibodies (MoAbs) against CD21, VLA-5, and MPC-1, and inhibition of adhesion of the immature type NOP-2 to KM102 by the anti-CD21 MoAb was observed as well. Furthermore, CD23 was detected on KM102. Treatment of KM102 with an anti-CD23 MoAb also inhibited adhesion of either KMS-5 or NOP-2 to KM102. Therefore, we propose that CD21 expressed on myeloma cells likely functions as a molecule responsible for the interaction of immature myeloma cells as well as mature myeloma cells with BM stromal cells, and CD23 may be the ligand on the stromal cells for the CD21-mediated adhesion.

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PLASMA CELLS AND their malignant counterpart, myeloma cells, mostly home to the bone marrow (BM) microenvironment. The BM stromal cells are believed to play a central role in the regulation of proliferation, terminal differentiation, and immunoglobulin production of myeloma and plasma cells. The interaction of myeloma cells with the BM stromal cells is believed to be mediated by the cell surface antigens called adhesion molecules. Myeloma cells have been reported to express several kinds of adhesion molecules, such as very late antigen 4 (VLA-4; CD49d), VLA-5 (CD49e), CD44, CD56, intercellular adhesion molecule-1 (ICAM-1; CD54), and lymphocyte function-associated antigen 1 (LFA-1; CD11a). Based on expression of the VLA-5 antigen, myeloma cells can be divided into mature (VLA-5+) and immature (VLA-5-) subpopulations. A cell surface antigen recognized by a monoclonal antibody (MoAb), MPC-1, was recently characterized to be a B-cell differentiation-associated antigen expressed on mature B cells and mature myeloma cells. Both the VLA-5 and MPC-1 antigens were reported to be involved in the adhesion of the mature myeloma cells to the BM stromal cells. However, myeloma cells from about one third of myeloma patients show the VLA-5' MPC-1' immature phenotype, yet these immature myeloma cells are rarely detected in the peripheral blood (PB). Also, we have observed that a myeloma cell line, NOP-2, which lacks expression of VLA-5 and MPC-1, nevertheless adheres to KM102. These phenomena imply that other adhesion molecules in addition to VLA-5 and MPC-1 may be responsible for the interaction of myeloma cells with BM stromal cells.

CD21 antigen was initially identified as a B-cell differentiation antigen expressed on mature B cells, serving as a receptor for C3d protein of the complement system and for gp350/220 envelope glycoprotein of Epstein-Barr virus (EBV). However, CD21 is not specific to B cells and is also expressed on some non-B lineage cells, such as T cells, follicular dendritic cells, and pharyngeal epithelial cells. The data from studies of the CD21 CDNA have shown that the extracellular domain of CD21 is formed by 15 to 16 short consensus repeats (SCRs), which are typical for the selectin adhesion molecule family. Recently, it was reported that CD21 can bind to a novel ligand, CD23. CD21 and CD23 are expressed on slgM+ slgD+ B cells and follicular dendritic cells of lymph nodes. The importance of CD23 in the differentiation of germinal center B cells into plasmablasts has been documented.

The purpose of the present study was to further clarify the mechanism of the interaction of myeloma cells, especially the immature myeloma cells, with BM stromal cells. We found that both mature and immature myeloma cells express CD21, and BM stromal cells express CD23. Thus, we investigated whether the expression of CD21 on myeloma cells is responsible for the interaction of myeloma cells with BM stromal cells, and we discuss the possible physiologic significance of CD21 on myeloma cells.

MATERIALS AND METHODS

Cells. KMS-5 and NOP-2 are human myeloma cell lines, established from an IgD-type myeloma patient and a Bence-Jones type

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(lambda light chain) myeloma patient.\(^{27}\) respectively, KM102 is a human BM stromal cell line, established by transfection of BM-adherent cell populations with a plasmid pSV3gpt DNA containing the coding sequence of the SV40 early region.\(^{28}\) The KM102 cells exhibit fibroblastic morphology in culture. The three cell lines, as well as Raji and Hela cell lines, were separately maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS). Both the KMS-5 and NOP-2 cell lines adhered to the KM102 when cultured.

Mononuclear cells were isolated from BM aspirates of myeloma patients and normal donors by Ficoll-Hypaque gradient centrifugation. Informed consent was obtained before BM aspiration procedure in all cases. Because myeloma cells and normal plasma cells are strongly positive for the CD38 antigen, we performed flow cytometric staining of the BM mononuclear cells using anti-CD38 MoAb for the identification of myeloma and plasma cells. Myeloma cells and normal plasma cells can be clearly distinguished on the histogram by flow cytometer from other types of cells that are immediately positive, weakly positive, or negative for CD38.\(^{3}\)

Primary BM stromal cells were prepared from BM samples of myeloma patients. The BM mononuclear cells isolated by Ficoll-Hypaque centrifugation were seeded into the wells of a 24-well plate (\(1 \times 10^6\) per well) and cultured in RPMI-1640 medium supplemented with 10% FCS. Nonadherent cells were washed off after 1 week. The culture was continued until the adherent cells (fibroblastic morphology) nearly reached confluency. The adherent cells (primary BM stromal cells) were immediately used for adhesion assay.

**MoAbs.** Anti-VLA-4/CD49d (HP2/21), anti-VLA-5/CD49e (SAM1), anti-CD44 (J-173), anti-CD56 (T199), anti-CD21 (BL13), and anti-CD19 (J4.119) MoAbs were purchased from Immunotech S.A. (Marseille, France). Anti-CD23 MoAb (B6) was purchased from Coulter Immunology (Hialeah, FL). Fluorescine isothiocyanate (FITC)-conjugated anti-CD38 (T16) and phycoerythrin (PE)-conjugated goat antirabbit IgG were purchased from Immunotech S.A. The MoAb MPC-1 was produced in our laboratory using the KMS-5 myeloma cell line as an immunogen. The MPC-1 antigen has been characterized to be a B-cell differentiation-associated antigen present on mature B cells, mature myeloma cells, and normal plasma cells, but absent on pre-B cells, germinal center B cells, and immature myeloma cells.\(^{19}\)

**Flow cytometry.** We performed single-color staining (indirect immunofluorescent staining) for cell lines (KMS-5, NOP-2, Raji, Hela, and KM102) and two-color staining (FITC/PE) for primary myeloma and plasma cells, as described previously.\(^{19}\) Briefly, KMS-5, NOP-2, Raji, Hela, or KM102 cells were stained with MoAb specific to CD21, CD23, VLA-5, MPC-1, VLA-4, CD44, or CD56, and then with PE-conjugated goat antirabbit IgG. Primary myeloma cells or normal plasma cells, after single-color staining (PE), were washed, blocked with normal mouse serum, and then stained with FITC-conjugated anti-CD38 MoAb. The cell surface immunofluorescence was evaluated by flow cytometry (Cytorn; Ortho Diagnostic System, Westwood, MA).

**Cell sorting.** BM mononuclear cells from myeloma patients were stained with anti-CD21 (PE) and anti-CD38 (FITC). The CD38\(^{+}\)/CD21\(^+\) myeloma cells were sorted by a cell sorter (Epics Elite; Coulter). The mRNA was extracted from the sorted myeloma cells for reverse transcription-polymerase chain reaction (RT-PCR).

**RT-PCR.** Total cellular RNA was extracted from cell lines (KMS-5, NOP-2, Raji, and Hela) with guanidinium thiocyanate followed by centrifugation in cesium chloride solution. For the sorted primary myeloma cells, mRNA was extracted directly from cell lysates by Oligotex-dT30 (Takara Biomedicals, Otsu, Japan) according to the manufacturer's instructions. The cDNA was prepared by reverse transcription (RT) at 37°C for 60 minutes in a 20-μL reaction mixture containing 2 μg of total RNA or mRNA purified from 2 × 10\(^6\) sorted myeloma cells, 100 pmol/L random hexamer, 0.5 mMol/L each 2-deoxyxynucleotide-5-triphosphate (dNTPs), 1X RT buffer (50 mMol/L Tris-HCl, pH 8.8, 7.5 mMol/L KCl, 3 mMol/L MgCl\(_2\)), 10 mMol/L dithiothreitol (DTT), 20 U of human placenta ribonuclease inhibitor, and 20 U of Superscript II reverse transcriptase (GIBCO BRL, Gaithersburg, MD). Amplification of the CD21 cDNA was performed using the following primers: 5'-TTTATCACGGATGTCAGGCA-3' (nucleotides (nt) 1511 to 1531) forward primer (5'-primer) and 5'-AACAGCAGTGAAGTCTTTGAA-3' (nt 2003 to 2023) reverse primer (3'-primer), designed according to the published sequence\(^{29}\) and synthesized by Toyobo Co, LTD (Osaka, Japan). Three microliters of cDNA were amplified in a 100-μL reaction mixture containing 1X PCR buffer (10 mMol/L Tris-HCl, pH 8.3, 50 mMol/L KCl, 1.5 mMol/L MgCl\(_2\), 0.01% gelatin), 50 μMol/L each dNTP, 200 mMol/L each primer, and 2 U of Taq polymerase (Takara Biomedicals). Thirty cycles of PCR were performed in a thermocycler (Astec Corp, Fukuoka, Japan) with the following conditions: 94°C, 1 minute; 55°C, 1 minute; and 72°C, 1 minute. Five microliters of PCR products were electrophoresed in a 1.5% agarose gel in Tris/boric acid/EDTA buffer. The gel was stained with ethidium bromide (EB) and photographed.

**Southern blot.** PCR products were electrophoresed and transferred onto a NITRAN filter (Schleicher & Schuell, Keene, NH). The filter was hybridized at 37°C overnight with a synthesized 30-mer oligonucleotide (5'-GCTCAGATCTCCATTTGCCAACATG-GATACA-3'), the sequence of which is consistent with that of nt 1802 to 1831 in CD21 cDNA.\(^{30}\) The probe was tailed with digoxigenin (DIG)-deoxyuridine triphosphate (dUTP) using a DIG Oligonucleotide Tail Kit (Boehringer Mannheim Biochemica, Mannheim, Germany). After being washed twice in 2X standard sodium citrate (SSC) containing 0.1% sodium dodecyl sulfate (SDS) at room temperature for 10 minutes and twice in 0.1X SSC containing 0.1% SDS at 40°C for 5 minutes, the filter was incubated for 30 minutes with anti-DIG-antibody Fab-fragments conjugated to alkaline phosphatase (Boehringer Mannheim Biochemica). Hybridized DNA was detected by incubating the filter with chemiluminescent reagent, AMPPD [3-[2-spiroadamantane]-4-methoxy-4-(3-phosphoryloxy)phenyl-1,2-dioxetane] and exposing it to Fuji x-ray films (Fuji Photo Film Co, LTD, Kanagawa, Japan).

**Adhesion assay.** Adhesion assay was performed as described previously.\(^{18}\) The KMS-5 or NOP-2 cells were inoculated together with saturating concentration of MoAb or control normal mouse IgG into a 24-well plate (1 × 10\(^4\) per well) in which KM102 cells (1 × 10\(^4\) per well) were seeded 1 day earlier. The plate was incubated for 3 hours at 37°C, and then the floating or weakly attached myeloma cells were harvested by gently shaking the plate and washing the wells twice. The bound cells, after being photographed, were detached and harvested by mechanically, strongly shaking the plate and washing the wells three times. It was observed under a phase contrast microscope that almost all myeloma cells were detached by such washing, whereas the stromal cell layers were not disturbed. The number of both adherent and nonadherent myeloma cells were separately counted on a hemocytometer under a microscope.

For the adhesion assay using the short-term cultured BM primary stromal cells, the KMS-5 cells (5 × 10\(^4\) per well) were inoculated together with MoAb or control normal mouse IgG into a 24-well plate that contained the nearly confluent primary stromal cells. The plate was incubated for 2 hours at 37°C. The floating and adherent KMS-5 cells were analyzed as described above. Percentage of myeloma cell adherence was calculated by the following formula:

\[
\text{% Adherence} = \frac{\text{No. of Adherent Myeloma Cells}}{\text{Total No. of Myeloma Cells}} \times 100
\]
or = \frac{(5 \times 10^9) - \text{No. of Nonadherent Myeloma Cells}}{5 \times 10^9 \times \text{Total No. of Myeloma Cells}} \times 100

The percent adherence thus calculated by the two formulae matched well.

RESULTS

Expression of CD21 on myeloma and plasma cells and CD23 on BM stromal cells. According to our previous studies, myeloma cells were phenotypically classified into VLA-5'MPC-1' mature and VLA-5'MPC-1' immature subpopulations, and the interaction of the mature myeloma cells with BM stromal cells was mediated by at least two molecules, VLA-5 and MPC-1. To further evaluate the adhesion mechanisms, we examined the expression of serial adhesion molecules (CD21, CD23, VLA-4, CD44, and CD56) on myeloma and plasma cells, as well as BM stromal cells. Myeloma cells from 30 myeloma samples were immunophenotyped using flow cytometry, and they were all CD19'CD56'. Among the 30 myeloma cases tested, there were 21 cases of myeloma cells with mature phenotype, and the other nine cases had immature phenotype. Flow cytometric staining showed that both mature and immature myeloma cells expressed CD21 on their surface (Fig 1).

Table 1 summarizes that CD21 was positive for myeloma cells in 26 of 30 cases, including eight cases of immature phenotype and 18 cases of mature phenotype. Like CD21, VLA-4, CD44, and CD56 were also detected on both mature and immature myeloma cells (Table 1), suggesting no correlation between their expression and maturity of myeloma cells. Normal plasma cells showed VLA-5'MPC-1' mature phenotype in all 10 normal cases, and all of them expressed CD21, VLA-4, CD44, and CD19, but lacked expression of CD56 (Fig 1 and Table 1). Neither myeloma cells nor normal plasma cells expressed CD23.

KMS-5 is a myeloma cell line with the VLA-5'MPC-1' mature phenotype, and NOP-2 is the other myeloma cell line with the VLA-5'MPC-1' immature phenotype. Figure 2 shows that both KMS-5 and NOP-2 expressed CD21, VLA-4, and CD44, but lacked expression of CD23. CD56 was detected on NOP-2 alone. The KM102 stromal cell line expressed all of the molecules: VLA-4, VLA-5, MPC-1, CD21, CD23, CD44, and CD56 (Fig 2).

To confirm the flow cytometric results, CD21 mRNA was amplified by RT-PCR. As shown in Fig 3, myeloma cell lines (KMS-5 and NOP-2) and primary myeloma cells expressed CD21 at the mRNA level.

Involvement of CD21 in the interaction of myeloma cells with BM stromal cells. To assess the role of CD21 in myeloma cell/stromal cell interaction, adhesion assays were performed using the myeloma cell lines KMS-5 and NOP-2 and the stromal cell line KM102, as well as primary stromal cells. KMS-5 represents the mature myeloma phenotype (VLA-5'MPC-1'), and NOP-2 represents the immature myeloma phenotype (VLA-5'MPC-1'). Figure 4A shows that anti-VLA-5 MoAb partially inhibited adhesion of KMS-5 to KM102, as did anti-MPC-1 MoAb, confirming our previous observation. As expected, adhesion of NOP-2 to KM102 was not inhibited by either anti-VLA-5 or anti-MPC-1 MoAb, because neither VLA-5 nor MPC-1 was expressed on NOP-2 (Fig 4B). CD21 was expressed on both KMS-5 and NOP-2. Anti-CD21 MoAb partially inhibited adhesion of both KMS-5 and NOP-2 to KM102 (Fig 4A through C). CD23 was expressed on KM102, and treatment of KM102 with anti-CD23 MoAb also partially inhibited adhesion of KMS-5 and NOP-2 to KM102 (Fig 4A through C).

To further verify the role of CD21, VLA-5, and MPC-1 in myeloma cell/stromal cell adhesion, antibody-blocking experiments were performed using KMS-5 and short-term

![Fig 1. Expression of CD21 on mature and immature myeloma cells and normal plasma cells. Mononuclear cells, freshly isolated by Ficoll-Hypaque gradient centrifugation from BM samples of a myeloma patient and a healthy donor, were stained with MoAb specific to VLA-5 (SAM1), MPC-1, CD21 (BL13), CD19 (J4.119), or CD56 (T199) and PE-conjugated goat antimouse IgG, and then with FITC-conjugated anti-CD38 MoAb (T16). The stained cells were analyzed by flow cytometry. The vertical axis shows PE-staining, and the horizontal axis shows FITC-staining. Myeloma cells (solid arrows) and normal plasma cells (dashed arrows) are recognized on the histograms according to their strong expression of CD38 antigen.](image-url)
Table 1. Expression of Adhesion Molecules on Myeloma and Plasma Cells

<table>
<thead>
<tr>
<th>Myeloma Cells</th>
<th>VLA-5</th>
<th>MPC-1</th>
<th>VLA-6</th>
<th>MPC-2</th>
<th>Plasma Cells: VLA-5</th>
<th>MPC-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD21</td>
<td>8 (60-100)</td>
<td>18 (60-100)</td>
<td>10 (65-100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLA-4</td>
<td>9 (80-100)</td>
<td>21 (80-100)</td>
<td>10 (80-100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD44</td>
<td>8 (85-100)</td>
<td>20 (85-100)</td>
<td>10 (85-100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD56</td>
<td>5 (92-100)</td>
<td>15 (92-100)</td>
<td>0 (6-0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD19</td>
<td>0 (0-5)</td>
<td>0 (0-5)</td>
<td>10 (90-100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD23</td>
<td>0 (0-3)</td>
<td>0 (0-3)</td>
<td>0 (0-3)</td>
<td></td>
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</tbody>
</table>

Data are expressed as number of positive cases, analyzed by two-color flow cytometry. The positivity was defined as over 10% of positive cells in CD38+ myeloma plasma cell fraction. Values in parentheses indicate percentage range of positive cells in CD38+ myeloma plasma cell fraction.

DISCUSSION

CD21 is a well-documented B-cell differentiation antigen expressed on mature B cells. Myeloma and plasma cells are believed to be in the terminal stage of the B-cell differentiation pathway. Some groups reported that CD21 was not detectable in myeloma and plasma cells using the anti-CD21 MoAbs HB-5 and B2.11,12 However, our flow cytometric data clearly show the expression of CD21 on the surface of primary myeloma and plasma cells, as well as myeloma cell lines using an anti-CD21 MoAb, BL13 (Figs 1 and 2). Furthermore, RT-PCR obviously amplified CD21 mRNA from myeloma cell lines and primary myeloma cells (Fig 3A). The amplified CD21 cDNA fragment (513-bp length) was probed by a synthesized oligonucleotide, the sequence of which is consistent with that of CD21 cDNA sense chain...
Fig 3. Amplification of CD21 mRNA by RT-PCR. Total RNA from KMS-5 and NOP-2 myeloma cell lines, Raji B cell line (positive control), and Hela cell line (negative control), and mRNA from sorted primary myeloma cells were reverse transcribed into cDNA and then amplified by PCR. The amplified products were electrophoresed in 1.5% agarose gel, stained with EB (A), and blotted with a CD21-specific oligonucleotide (B). A 513-bp band is seen in the samples of Raji (lane 1), KMS-5 (lane 2), NOP-2 (lane 3), and primary myeloma cells (lane 4), but not in that of Hela (lane 5). The mRNA extracted from five cases of primary myeloma cells was amplified by RT-PCR. Same results were obtained as that shown in lane 4. The Hae III DNA size markers are shown in lane M.

and sites between the 5'-primer and 3'-primer (Fig 3B), indicating the specific amplification of CD21 mRNA by RT-PCR. CD21 has a long extracellular domain consisting of repetitive consensus units of 60 to 70 amino acids, termed SCRs. Two forms of CD21 cDNA (15 SCRs and 16 SCRs) have been cloned from human tonsil cells. They differ by a single SCR of 177 bp in length. Most of tonsillar B cells express CD21 with the 15 SCRs, while a few tonsillar B cells and the Raji B cell line express CD21 with the 16 SCRs. We designed PCR primers flanking the sequence containing the additional 177-bp SCR. Thus, if myeloma cells express CD21 with the 16 SCRs, the length of the PCR fragment would be 690 bp; otherwise, 513 bp. The results were that myeloma cells, myeloma cell lines, and even the Raji cell line consistently expressed CD21 with the 15 SCRs, suggesting that B cells and myeloma (plasma) cells commonly expressed the 15-SCR CD21 molecule. However, the question may be raised about the difference in reactivity among the anti-CD21 MoAbs. It is possible that BL13 may recognize an epitope on CD21 distinct from that recognized by HB-5 or B2.

Stromal cells express many kinds of adhesion molecules. Our data showed that the KM102 stromal cell line expressed VLA-4, VLA-5, CD21, CD23, CD44, CD56, and MPC-1. This is reasonable in stromal cells, because many types of cells in BM interact with stromal cells. It is interesting that CD23 is expressed on KM102. This finding prompted us to consider that CD21 may function as an adhesion molecule in myeloma cell/stromal cell interaction, because CD23 is a ligand for CD21. Thus, adhesion assays were performed using myeloma cell lines (KMS-5 and NOP-2) and a stromal cell line (KM102), as well as primary stromal cells.

KMS-5 was positive for VLA-5 and MPC-1, and was
thus considered representative of mature type. NOP-2 was negative for VLA-5 and MPC-1, so it was representative of immature type. Both KMS-5 and NOP-2 expressed CD21. Adhesion of either KMS-5 or NOP-2 to KM102 was inhibited by the anti-CD21 MoAb, BL13. Adhesion of KMS-5 (not NOP-2) to KM102 was also inhibited by anti-VLA-5 and anti-MPC-1. Further confirmation was made by adhesion assay performed in KMS-5 versus primary BM stromal cells.

Each of the MoAbs to CD21, VLA-5, and MPC-1 could inhibit adhesion of KMS-5 to primary BM stromal cells. In addition, treatment of KM102 with anti-CD23 MoAb inhibited adhesion of KMS-5 and NOP-2 to KM102. Therefore, in addition to VLA-5 and MPC-1, CD21 could serve as another adhesion molecule for the interaction of the mature myeloma cells with BM stromal cells. Notably, CD21 is also involved in the interaction of the immature myeloma cells.
with BM stromal cells. The CD21-mediated myeloma cell/stromal cell interaction may be through its binding to CD23 on stromal cells.

In contrast to the MoAbs specific to CD21, VLA-5, or MPC-1, anti-VLA-4 MoAb (HP2/1) and anti-CD44 MoAb (3-173) did not inhibit, but rather enhanced the adhesion of KMSJ and NOP-2 to KM102. The HP2/1 MoAb has been reported to inhibit the binding of VLA-4 to its two ligands, fibronectin (FN) and vascular cell adhesion molecule-1 (VCAM-1), and also to inhibit the adhesion of myeloma cell lines to FN. Interestingly, the other anti-VLA-4 MoAbs (HP2/4, L25, and P4C2) have been shown not only to inhibit the binding of B cells to FN and VCAM-1, but also to trigger cell aggregation. The nature of anti-VLA-4--triggered adhesion is unknown. Anti-CD44--triggered adhesion has also been observed in follicular dendritic cell/B-cell adhesion and homotypic T-cell aggregation. In those studies, the promotion of cell aggregation by an anti-CD44 MoAb, NK1-P2, is through the enhancement of LFA-1 expression. However, for myeloma cells, further analysis is needed to clarify the mechanism.

Normal plasma cells can be phenotypically distinguished from myeloma cells based on differential expression of CD19 antigen: normal plasma cells express CD19, whereas most myeloma cells lack its expression. It has been reported that CD21 is associated with CD19 on the cell surface of human B lymphocytes. In vitro signal transduction can be mediated by triggering CD21 on B cells with soluble CD23 or EBV protein. However, in view of the relatively short cytoplasmic tail of CD21, CD19 may function as a signal transduction unit for the CD21-mediated events. To date, there is no evidence to show whether the CD21-CD19 complex is present in plasma cells and absent in myeloma cells, and if so, whether signal events through the CD21-CD19 complex are different between myeloma cells and plasma cells. Studies are now being conducted to answer these questions.

In summary, both mature and immature myeloma cells,
as well as normal plasma cells, express CD21. CD21 may function as one of the adhesion molecules for the interaction of both mature and immature myeloma cells with BM stromal cells. The CD21-mediated adhesion may be through its binding to CD23 on stromal cells. The CD21/CD23 pathway is important for the interaction of myeloma cells, especially the immature myeloma cells, with BM stromal cells.

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Table 2. Inhibition of KMS-5 Adhesion to Primary BM Stromal Cells

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>% Adherence</th>
</tr>
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<tr>
<td>Control</td>
<td>73.0 ± 1.0</td>
</tr>
<tr>
<td>Anti−CD21</td>
<td>35.3 ± 1.1</td>
</tr>
<tr>
<td>Anti−VLA-5</td>
<td>30.7 ± 1.5</td>
</tr>
<tr>
<td>Anti−MPC-1</td>
<td>40.0 ± 0.9</td>
</tr>
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</table>

Percent adherence was calculated as described in Materials and Methods. Data are expressed as the mean ± SD of triplicate wells. Experiments 1, 2, and 3 represent adhesion assays performed on stromal cells obtained from patients 1, 2, and 3, respectively. Control was made by substituting normal mouse Ig for MoAbs.

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