CD11a-CD18 and CD102 Interactions Mediate Human Myeloma Cell Growth Arrest Induced by CD40 Stimulation

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ABSTRACT

We have recently demonstrated that the CD40 molecule was expressed on both normal human plasma cells and most malignant plasma cells, i.e., myeloma cells. Thus, we have investigated its putative role in the proliferation of myeloma cells. We report that 7 of 15 myeloma cell lines were CD40+ but only one, XG2, presented a high level of CD40 expression. We show that the CD40 stimulation by anti-CD40 monoclonal antibodies (mAbs) of the interleukin 6-dependent myeloma cell line XG2 induced a total inhibition of its proliferation. This inhibition was observed when cells were either cultured in the “CD40 system,” where the anti-CD40 mAb has been immobilized on fibroblasts expressing Fc receptors or in the presence of a soluble chimeric CD40 ligand molecule. This inhibition of proliferation was neither accompanied by differentiation nor apoptosis. Triggering CD40 induced an homotypic aggregation of XG2 cells, and the inhibition of proliferation was totally prevented by a blocking anti-CD18 mAb. Although the CD11a-CD18 ligands, i.e., CD50, CD54, and CD102, were all expressed on XG2 cells, only a blocking anti-CD102 mAb inhibited the CD40-induced growth arrest. Our data demonstrate that CD40 triggering on XG2 cells induced a myeloma cell growth arrest mediated by lymphocyte function-associated antigen 1 and intercellular adhesion molecule 2 interactions.

INTRODUCTION

The CD40 molecule is a Mr 48,000 glycosylated phosphoprotein originally identified in B lymphocytes (Bp50) as well as in some B-cell malignancies and carcinomas (1). CD40 antigen has also been detected on activated macrophages, dendritic cells, and Langerhans cells (2). The CD40 molecule belongs to the superfamily of the TNF receptor which includes CD30, NGF-R, CD27, and Fas (1, 3). This TNF receptor family interacts with a parallel family of ligands including the CD40L or gp39 (3, 4). The CD40L expression has essentially been described on activated T lymphocytes (5–7). On B cells, CD40 has been described extensively as a molecule essential for both proliferation and differentiation (for review, see Ref. 2). By itself, stimulation through CD40 can prevent germinal center B-cell apoptosis induced by antigen receptor cross-linking (8–10); in combination with IL-4, it can trigger B-cell proliferation (11) and immunoglobulin secretion (12), and, in conjunction with IL-10, it can induce B-cell differentiation into plasma cells (13). Nevertheless, recent data indicate that CD40 signaling may not always deliver an anti-apoptotic activation but on the contrary may lead to a Fas-mediated apoptosis sensitivity (14) or to a cell growth arrest (15). All of these data demonstrate that the B-cell response to CD40 stimulation depends on the stage of differentiation or activation of B lymphocytes.

We have recently demonstrated that CD40 was expressed on both normal and malignant plasma cells (16). MM is a malignancy characterized by an accumulation of malignant clonal plasma cells within the bone marrow. MM is often regarded as a B-cell malignancy, although myeloma cells are clearly more differentiated than the germinal center or resting B cells since they do not express the same surface differentiation antigens, do not respond to the same cytokines, and secrete immunoglobulin: myeloma cells have lost most of the B-cell antigens (surface immunoglobulin, CD20, CD21, CD80, HLA-DR, etc.) but express antigens like B-B4, CD28, and CD56 that have not been found on B cells (16, 17). On the contrary, CD40 is an antigen expressed throughout B-cell differentiation, including both normal and malignant plasma cells (2, 16). Because of the role of CD40 in B-cell proliferation, the expression of this antigen on normal plasma cells, which are terminally differentiated and nonproliferative cells, was somewhat surprising. We have found that CD40 was also expressed on approximately 80% of 50 patients with MM and on 7 of 12 myeloma cell lines (16, 17). The presence of CD40 on malignant plasma cells allowed us to hypothesize its involvement in the proliferation of myeloma cells. It is clearly demonstrated that IL-6 is the major cytokine supporting myeloma cell proliferation (18, 19). In the murine B-lymphoma cell line M12 transfected with human CD40, triggering CD40 led to IL-6 secretion (20). Westendorf et al. (21) have reported that stimulation of CD40 in one IL-6-dependent human myeloma cell line, ANBL-6, induced IL-6 secretion and, therefore, proliferation. This finding indicated that myeloma cell proliferation could be supported by an autocrine IL-6 secretion induced by CD40 triggering. In the laboratory, nine myeloma cell lines called XG1–XG9 have been established, the growth of which was dependent on the exogenous addition of IL-6 (17). Among these cell lines, five of nine were CD40+ but only one, XG2, presented a high staining for CD40 antigen, whereas the other four lines were only weakly positive. In this report, we have investigated the consequences of CD40 triggering on myeloma proliferation, and we demonstrated that it induced the growth arrest of XG2 cells.

MATERIALS AND METHODS

Reagents. The anti-CD40 IgG1 mAbs (mAbB9 and B-B20) and the CD40L were kindly provided by Dr. J. Blanchereau (22), Dr. J. Wijdenes (Diaclone, Besançon, France), and Dr. D. Hollenbaugh (4), respectively. FITC-treated, PE-treated control IgG1, FITC-treated anti-CD28, and FITC-treated anti-CD54 mAbs were obtained from Immunotech (Marseille, France) and PE-treated anti-CD11a and FITC-treated anti-CD18 mAbs were obtained from DAKO (Glostrup, Denmark). Anti-CD80 mAb (B-B1) was obtained from Becton Dickinson; anti-CD86 mAb (BU63) and anti-CD102 mAb (6D5) were kindly provided by Dr. D. Hardie (Birmingham, United Kingdom) and Dr. C. G. Gahmberg (Helsinki, Finland), respectively. Biotinylated of purified mAbs was performed as previously described (16). For functional studies, we used the following purified mAbs: anti-CD18 (7E4) was obtained from Immunotech, anti-CD28 (CD28.5) was kindly provided by Dr. D. Olive (Marseilles, France), and anti-CD50, anti-CD54, and anti-CD102 were mAbs submitted to the Vth International Conference on Human Leukocyte Differentiation Antigens.

Flow Cytometric Analysis of Myeloma Cell Lines. For immunofluorescence staining, 5 × 10^6 cells were incubated with 0.5 µg anti-CD40 mAb or control IgG1 mAb for 30 min at 4°C, followed by a secondary incubation with 0.5
μg FITC-goat antimouse for 30 min at 4°C. Cells were fixed in 4% formaldehyde and then analyzed on a FACScan flow cytometer (Becton Dickinson). The results were expressed as the MFI, corresponding to the specific fluorescence intensity divided by the control staining.

For immunofluorescence staining after CD40 triggering, directly conjugated mAbs were used: FITC-control IgG1, PE-control IgG1, PE-CD11a, FITC-CD18, FITC-CD28, FITC-CD4, and biotinylated CD54 (CBR-IC3/5), CD80, CD86, and CD102 (CBR-IC2/2). Bound biotinylated mAbs were then revealed with PE-streptavidin (Immunotech).

Myeloma Cells Lines Culture and Proliferation Assays. XG myeloma cell lines were maintained in RPMI 1640 (Life Technologies, Inc., Cergy-Pontoise, France) supplemented with 5% heat-inactivated FCS (Life Technologies, Inc.), 2 mM glutamine, 100 μM streptomycin, 100 units/ml penicillin, 2 x 10^{-3} M 2-mercaptoethanol, and 3 ng/ml IL-6 (17). RPMI 8226 and U266 cell lines were purchased from American Type Culture Collection, and LP1, L363, NCI-H929, and OPM2 were purchased from DSM (Braunschweig, Germany); all of these cell lines were maintained in medium without IL-6. For XG proliferation assays, IL-6 was removed by extensive washes followed by a 6-h preincubation in RPMI 1640 without IL-6. Cultures were incubated for 3 to 4 days at 37°C in a 5% CO2 humidified atmosphere. Then 1 μCi [3H]thymidine (specific activity, 20 Ci/mmol; NEN Dupont de Nemours, Les Ulis, France) was added during the last 18 h. The cells were lysed with a Skatron cell harvester, transferred to a glass filter, and [3H]thymidine incorporation was quantitated by liquid scintillation spectroscopy. The results were expressed as the mean of triplicate wells ± SD.

Preparation of Peripheral B Cells. B cells were purified from peripheral blood: mononuclear cells were isolated by Ficoll-Hypaque centrifugation, monocytes were removed by adherence to culture dishes, and T cells were eliminated by sheep erythrocytes rosetting.

IL-6 Determination Using ELISA. XG2 cells were extensively washed, preincubated for 6 h in medium without IL-6, and cultured for 3 days in RPMI 1640 with 5% FCS at the cell density of 10^5 cells/ml with or without anti-CD40 mAb (1 μg/ml) or CD40L (dilution 1:16). Secreted IL-6 was determined using the ELISA (Innoteq, Besançon, France). This test allowed the detection of 15 pg IL-6.

Culture of Myeloma Cells and B Cells on CD32L Fibroblasts. XG cells or B lymphocytes were cultured in the presence of anti-CD40 mAb89 presented by the mouse fibroblastic Ltk- cell line stably expressing CDw32 (11).

Measurement of Immunoglobulin Secretion. XG2 cells (5 x 10^5 cells/ml) were cultured for 4 days with or without 1 μg/ml anti-CD40 mAb89. Secreted immunoglobulin was measured using a specific ELISA. In brief, 0.5 μg mouse antihuman κ or λ antibody (Immunotech) was coated on 96-wells ELISA plates (Nunc). After incubation with cellular supernatants, the amount of κ or λ bound was measured by a goat antihuman IgH or λ antibody (GAH) directly coupled to peroxidase (Immunotech), and the quantity of GAH bound was revealed then by the oxidation of o-phenylenediamine dihydrochloride (Sigma), determined by the absorbance at 490 nm. The test was standardized with purified IgGκ and IgGλ (Sigma) between 0.5 and 500 ng.

Cell Cycle and BrdUrd Incorporation Analysis. XG2 cells (5 x 10^5 cells/ml) were cultured for 3 days with 75 pg/ml IL-6 with or without 2 μg/ml B20 mAb. BrdUrd incorporation (5 μM) was performed the last 2 h before harvesting the cells. Cells were washed and fixed overnight at 4°C in 1% paraformaldehyde containing 0.01% Tween 20, as described (23). Cell pellets were washed and incubated for 30 min at 37°C with 4 μg/ml bovine DNase I in PBS containing Ca^{2+} and Mg^{2+}, then washed in PBS/0.5% Tween 20 and incubated for 45 min with FITC-conjugated anti-BrdUrd BU-5 mAb (Euromedex; Souffelweyersheim, France) as well as washed and incubated for 10 min with PI (DNAprep, Coulter counter).

RESULTS

CD40 Expression Was Heterogenous on Myeloma Cell Lines. Nine myeloma cell lines (XG1-XG9), the growth of which was dependent on exogenous IL-6 were established in the laboratory (17). CD40 was expressed on five of these XG cell lines: XG2, 3, 4, 6, and 9. Except for XG2, which was highly positive for CD40, the level of CD40 staining of the four other positive cell lines was very weak (illustrated by XG6 in Fig. 1), while the others were totally negative (illustrated by XG1, Fig. 1). Among the six exogenous IL-6-independent myeloma cell lines we have tested, i.e., NCI-H929, LP1, L363, OPM2, RPMI 8226, and U266, only LP1 and RPMI 8226 were CD40+ and the intensity of staining was weak (illustrated by RPMI 8226, Fig. 1). In summary, seven MM cell lines were CD40+ (XG2, 3, 4, 6, 9, LP1, and RPMI 8266) and eight MM cell lines were CD40- (XG1, 5, 7, 8, NCI-H929, L363, OPM2, and U266); actually only one, XG2, was highly positive for CD40 and by consequence was the major purpose of this study.

Anti-CD40 mAbs (mAb89 and B20) Inhibited the Growth of XG2 Myeloma Cell Line. To determine the possible role of CD40 on XG myeloma cell proliferation, we cultured XG2 and XG6 cells with 1 μg/ml anti-CD40 mAb89 in the absence or presence of increasing concentrations of IL-6. As shown in Fig. 2A, with or without exogenous IL-6, 1 μg/ml anti-CD40 mAb dramatically inhibited the proliferation of XG2: from 7.5 to 750 pg/ml IL-6, the growth inhibition ranged from 80% to 70%. With regard to the XG6 cell line, in which cells were only weakly positive for CD40, the proliferation was not modified by anti-CD40 mAb (Fig. 2B). As a control, we used an anti-CD28 mAb (CD28.5) because all myeloma cell lines are CD28- (16, 17); the presence of 1 μg/ml CD28.5 did not modify the proliferation of myeloma cell lines.

To compare the effects of two anti-CD40 mAbs (mAb89 and B20), XG2 cells were cultured with growing concentrations of each antibody in the presence of 75 pg/ml IL-6. The inhibition of proliferation induced by both anti-CD40 mAbs was detectable with only 0.1 μg/ml (61.3% ± 1.2% growth inhibition with 0.1 μg/ml mAb89). The mAb89 was more potent than B20 since the inhibition of proliferation induced by mAb89 was significantly more pronounced than the one induced by B20 (for 1 μg/ml, the inhibition was of 86% ± 5% and 47.5% ± 8.8% respectively; Fig. 2C). With 10 μg/ml mAbs, the percentage of inhibition was unchanged for mAb89 but slightly enhanced for B20 (60% ± 1.1%).
Proliferation of XG2 was inhibited in the “CD40 System” and by a Soluble CD40L. The capacity of anti-CD40 mAb to induce B-cell proliferation is highly different when the mAb is in a soluble form or immobilized. Indeed, Banchereau et al. (11) have described an illimited B-cell proliferation obtained with IL-4 and anti-CD40 mAb (mAb89) presented by Fc receptors on CD32-transfected fibroblasts. This culture system, called the CD40 system, seems to mimic the action of activated helper T lymphocytes which express CD40L and secrete IL-4 (23, 24). Fig. 3 shows the results obtained when XG2 cells were cultured in the CD40 system: with or without 100 pg/ml IL-6, similar to what is observed with soluble anti-CD40 mAb89, mAb89 still strongly inhibited XG2 proliferation (70% inhibition with 1 μg/ml mAb89). The addition of 100 units/ml IL-4 did not significantly affect the inhibition induced by anti-CD40 mAb. To ensure our experimental conditions, we cultured peripheral blood B cells in the same CD40 system. As shown in Fig. 3B, 1 μg/ml anti-CD40 mAb in the presence of 100 units/ml IL-4 stimulated thymidine incorporation of B lymphocytes: the index of stimulation was 520 compared to that of control. With regard to XG6, the immobilization of anti-CD40 mAb89 or B-B20 on fibroblasts did not allow any inhibition of its proliferation (Fig. 3C).
did not detect any IL-6 secretion by the XG2 cells: no detectable IL-6 was found in XG2 supernatant and no IL-6 mRNA was detected with Northern blot in XG2 cells (data not shown).

Anti-CD40 mAb Induced a Growth Arrest without Differentiation or Apoptosis. To determine whether the growth arrest was accompanied by differentiation, we measured the amount of immunoglobulin secreted. XG2 cells routinely produced 0.5 ng of IgGA/10^6 cells/day in the presence of 1 ng/ml IL-6. In the absence of IL-6, XG2 cells did not proliferate and the amount of immunoglobulin secreted decreased in parallel (20—30%). The addition of 1 µg/ml anti-CD40 mAb slightly (10—15%) reduced the quantity of immunoglobulin secreted in the presence or absence of IL-6. Therefore, based on immunoglobulin secretion, no differentiation of XG2 was observed in the presence of anti-CD40 mAb.

To characterize the inhibition of proliferation, we analyzed the BrdUrd incorporation and the DNA content using a double-fluorescence staining method (25). Cell cycle analysis and BrdUrd incorporation revealed that anti-CD40 mAb-treated cells were blocked in the S-phase. Fig. 5 shows that the percentage of BrdUrd-positive cells (upper right quadra) was 44% in the control (A) and 28% in the B-B20-treated cells (B), indicating a reduction of the S-phase. The proportion of cells in G2 (Fig. 5, A and B, R2 region) was reduced from 3.4% to 2%. DNA content showed that cells were still in the S-phase but BrdUrd staining revealed that these cells did not incorporate BrdUrd anymore, indicating a cell growth arrest in the S-phase. In the population of anti-CD40 mAb-treated cells, we did not detect apoptotic cells which are characterized by a diminution of PI intensity associated with a reduced cell size: the dot plots in Fig. 5, C and D, showed that no apoptotic cells were detected after 72 h with anti-CD40 mAb. The same results were obtained after 24, 48, and 96 h (data not shown). Furthermore, no fragmented DNA could be seen (data not shown). This growth arrest in the S-phase was already

Neither Anti-CD40 mAb nor CD40L Induced IL-6 Secretion. Westendorf et al. (21) reported that an anti-CD40 mAb (G28.5) induced IL-6 secretion in the myeloma cell line ANBL-6. In the presence or absence of 1 µg/ml mAb89 or CD40L (dilution 1:16), we did not detect any IL-6 secretion by the XG2 cells: no detectable IL-6 was found in XG2 supernatant and no IL-6 mRNA was detected with Northern blot in XG2 cells (data not shown).

Fig. 4. Cell growth inhibition of XG2 cells by soluble CD40L. XG2 cells were cultured with increasing dilutions of soluble CD40L containing supernatant or control supernatant (supernatant of untransfected COS cells) in the presence of 75 pg/ml IL-6. Proliferation was measured by [3H]thymidine incorporation at day 4. Results are expressed as the percentage of control (without COS supernatant) and are the means of triplicate wells. Bars, SD. Representative experiment of four.
observed after 48 h (38% of the cells in the S-phase in the control population and 31% in the B-B20-treated population). We concluded that anti-CD40 mAb induced a cell growth arrest without differentiation or apoptosis.

Triggering CD40 on Myeloma Cells Did Not Induce CD80 Expression nor Enhance the Constitutive Expression of CD86. Previous studies have demonstrated that CD40 stimulation on normal or leukemic B cells induced CD80 expression and allowed cell cooperation between T and B lymphocytes (26). Because we previously demonstrated that some malignant plasma cells and all of the myeloma cell lines we have tested expressed CD28 (16), we were interested in determining whether myeloma cell lines coexpressed the CD28 ligands, namely, B7.1 (CD80) and B7.2 (CD86). In contrast to B cells, no CD80 expression was found on myeloma cell lines, and no induction of CD80 expression was observed on XG2 after CD40 triggering (Fig. 6). On the contrary, we found that all 15 myeloma cell lines were weakly CD86+ (3 < MFIR < 10, MFIR = 3 for XG2), but no stimulation of CD86 expression was observed on XG2 after CD40 triggering (Fig. 6). CD28 expression also (MFIR = 5) was not modified when XG2 cells were stimulated with anti-CD40 mAb.

Anti-CD40 mAb-Induced Cell Aggregation. It has been previously described that stimulation of CD40 on B cells led to homotypic aggregation involving the adhesion molecules LFA-1 (CD11a-CD18) and one of its counterreceptors, ICAM-1 (CD54; Ref. 27). In Fig. 7, we show that stimulation of XG2 by anti-CD40 mAb89 (or B-B20; data not shown) induced cell aggregation that was already detectable after 24 h. Myeloma cell lines are mainly CD11a+/CD18− (17). Of note, XG2 and RPMI 8226 were the only CD11a+/CD18+ myeloma cell lines in our hands among the 15 MM cell lines listed above. As shown in Fig. 6, XG2 was weakly CD11a+/CD18+ (MFIR = 7) and expressed the three known ligands of LFA-1, i.e., ICAM-1 (CD54), ICAM-2 (CD102), and ICAM-3 (CD50) with a MFIR of 35, 25, and 8, respectively. The stimulation of CD40 did not up-regulate the level of expression of any of these four molecules (Fig. 6).

Anti-CD18 and Anti-CD102 mAbs Prevented CD40-Induced Inhibition of Proliferation. The addition of a blocking anti-CD18 mAb (7E4) reduced the cell aggregation and the inhibition of proliferation induced by CD40 triggering on XG2 cells: 1 µg/ml B-B20 induced an inhibition of proliferation of 64% ± 5% that was reduced to 20% ± 2.6% with 5 µg/ml 7E4 mAb (Fig. 8A). In the presence of a blocking anti-CD54 mAb (YH370), the inhibition of proliferation induced by B-B20 was not significantly modified: the B-B20-induced inhibition was 64% ± 5% or 73% ± 3% with or without 10 µg/ml YH370, respectively (Fig. 8A).

Since anti-CD54 mAb did not block the growth arrest induced by CD40 triggering, we looked further for the possible involvement of the two other LFA-1 ligands, CD102 and CD50 (28, 29). The mAbs used were those submitted to the Vth International Conference on Human Leukocyte Differentiation Antigens. Whereas none of the five anti-CD50 mAbs modified CD40-induced growth arrest, one of the two anti-CD102 mAbs totally prevented CD40-induced growth arrest (Fig. 8B): compared to control, the residual growth was 38% ± 6.7% in the presence of 1 µg/ml B-B20 mAb but was enhanced to 87% ± 10.3% or even to 94.6% ± 8.9% by the addition of 10 or 20 µg/ml 6D5 mAb, respectively. Blocking CD18 with 7E4 mAb or CD102 with 6D5 mAb on unstimulated XG2 cells did not modify the proliferation (data not shown).

**DISCUSSION**

In this study, we have shown that 7 MM cell lines of 15 were CD40+ but only one, XG2, presented a high expression of CD40 while the other remained weakly positive. Therefore, we can conclude...
that CD40 was not expressed on all MM cell lines but taken in conjunction with ANBL-6 (21), on only 8 of 16. We have demonstrated that the CD40 triggering by anti-CD40 mAbs or CD40L of the highly CD40+ myeloma cell line XG2 induced an inhibition of its proliferation, while the proliferation of myeloma cell lines weakly positive for CD40 was insensitive to CD40 triggering (XG6, XG4). The triggering of CD40 on XG2 cells did not induce differentiation or apoptosis but a cell growth arrest characterized by a stop in the S-phase. Furthermore, we have shown that CD40 stimulation induced an homotypic aggregation of XG2 cells. This aggregation was not observed with the other CD40+ myeloma cell lines, indicating that aggregation and cell growth arrest were positively correlated. It has been previously shown that stimulation of CD40 induced an homotypic adhesion of B lymphocytes: Barrett et al. (27) demonstrated that LFA-1, but not ICAM-1, was involved since anti-CD18 mAb but not anti-CD54 mAb inhibited CD40-induced cell aggregation and cell response. Although LFA-1 and ICAM-1 were found to be expressed on XG2 cells, no spontaneous aggregation was observed. Furthermore, during the CD40-induced aggregation, we did not observe any strong increase in the levels of LFA-1 or ICAM-1 expression that could explain this aggregation. These data are in good agreement with those of Hibbs et al. (30), who demonstrated that LFA-1 was not constitutively avid for its counterreceptors ICAM-1 and -2 but was converted into a high-affinity state upon cellular activation. The avidity of LFA-1 was mediated by the β subunit, CD18, and was probably regulated by phosphorylation (30, 31). We demonstrated that blocking anti-CD18 but not anti-CD54 mAbs prevented CD40-induced growth arrest, indicating that activation of LFA-1 was required. Since other ligands have been described for LFA-1, i.e., ICAM-2 (CD102) and -3 (CD50), we looked for the possible involvement of these molecules in the CD40-induced cell growth arrest. We have observed that myeloma cell lines expressed CD50 and CD102, and we have demonstrated that one anti-CD102 mAb, 6D5, totally prevented CD40-mediated inhibition of proliferation.

The 6D5 mAb has been shown to inhibit the adhesion of LFA-1+ leukocyte (HL60) to ICAM-2-transfected COS-1 cells (32). Although no anti-CD50 mAbs among five interfered with CD40-induced inhibition, we cannot exclude the involvement of CD50, since these mAbs have not been characterized as blocking or not blocking mAbs. Altogether, our data indicate that activation of both LFA-1 and its counterreceptor ICAM-2 was required for CD40-induced myeloma cell growth arrest and support the hypothesis that this activation was mediated by direct interactions between these two counterreceptors. Although the involvement of LFA-1 in the homotypic aggregation and
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in the biological response of B cells to CD40 triggering has been previously reported, our data represent the first evidence that cell response to CD40 triggering in terms of proliferation involves LFA-1 and ICAM-2 interactions (27, 33).

In the ANBL-6 myeloma cell line, CD40 stimulation by the anti-CD40 mAb G28.5 or cells expressing CD40L triggered IL-6 secretion and then cell proliferation (21). With regard to XG2, CD40 stimulation did not induce any secretion of detectable amounts of IL-6 and any proliferation of cells, that was even still inhibited in the presence of exogenous IL-6. Taking all of these data together, we can conclude that myeloma cell lines are heterogenous with regard to CD40-induced IL-6 secretion and cell response. Such heterogeneity was also observed with regard to both the membrane molecule expression such as CD40, CD56, and LFA-1 and the cell response to the other gp130 family cytokines, such as leukemia inhibitory factor and oncostatin M (16, 17, 34). This heterogeneity of MM cell lines might precisely explain the opposite consequences of CD40 triggering on ANBL-6 and XG2 proliferation. In fact, ANBL-6 did not express LFA-1 (35), and since we have demonstrated that CD40-induced growth arrest of XG2 was blocked by anti-CD18 mAb, it is tempting to speculate that the lack of LFA-1 expression on ANBL-6 might explain why this cell line was not inhibited upon CD40 stimulation.

Although normal and malignant plasma cells expressed CD54, we recently demonstrated that LFA-1 was strongly underexpressed on myeloma cells compared to normal plasma cells (36, 37). Indeed, the mean percentage of LFA-1* plasma cells was 66% for normal plasma cells (10 donors) but only 23% for malignant plasma cells (48 patients). Furthermore, the percentage of LFA-1* myeloma cells was all the more weak because the patients were in progressive and terminal phases of the disease (37). Therefore, LFA-1 was rarely expressed on myeloma cell lines, only on 2 of 15, i.e., XG2 and RPMI 8226. RPMI 8226 weakly coexpressed CD40 and LFA-1, and we observed that CD40 triggering induced a partial inhibition of proliferation (data not shown). Taken together, our data could support the hypothesis that the responsiveness of myeloma cell lines to CD40 triggering in terms of growth arrest was related to the CD40 level of expression or to the expression of LFA-1, or both.

Our data indicating an underexpression of LFA-1 on myeloma cells contrasts with the previous report of Ahsmann et al. (38), who demonstrated the existence of a correlation between tumor growth and LFA-1 expression. In this study, the expression of LFA-1 was determined by a simple staining of bone marrow cells without prior purification of plasma cells. In our study, we have determined the phenotype of normal and malignant plasma cells by a double staining with CD38 or B-B4, allowing a specific identification of plasma cells (16, 37). Our finding that LFA-1 was all the less expressed because the patients were in progressive and terminal phases of the disease was in good agreement with the general correlation described between the lack of LFA-1 and the aggressiveness of lymphomas, and with the general hypothesis that the absence of LFA-1 on lymphomas favors the escape of the tumor from the immune surveillance (39, 40).

Recently, Tong et al. (41) reported that CD40 triggering on myeloma cell lines enhanced the clonogenicity but excluded the involvement of IL-6 secretion. On the contrary, Urashima et al. (42) reported that CD40L triggered IL-6 secretion in multiple myeloma and stimulated proliferation. The conclusions expressed in these two articles were essentially based on the data obtained with lymphoblastoid cell lines inappropriately called myeloma cell lines (ARH-77, HS-Sultan, and IM-9). In fact, these cell lines have been described clearly as lymphoblastoid cell lines transformed by the EBV: they do not present the phenotypic and karyotypic characteristics of the myeloma cell lines. These three cell lines express the B-cell antigens CD19, CD20, and CD21, surface immunoglobulins that are not found on MM cell lines but on the contrary do not express molecules always found on myeloma cell lines, CD28 and B-B4 (43). This inappropriate utilization of such cell lines as a model for multiple myeloma had already been outlined 9 years ago by Gazdar et al. (44), who clearly demonstrated that HS-Sultan and ARH-77 were indistinguishable from lymphoblastoid cell lines. The authors explained that they were identified as myeloma cell lines because they were obtained from the culture of myeloma patients' bone marrows. In fact, the lymphoblastoid cell lines also spontaneously emerged from normal bone marrow cultures due to the immortalization induced by EBV infection (45).

Therefore, based on data obtained with MM cell lines XG2 and ANBL-6, we can conclude that cell response to CD40 triggering is heterogenous in terms of proliferation, and this heterogeneity may be support by alternative LFA-1 expression.

In MM, it is clearly demonstrated that IL-6 is the major growth factor of myeloma cells in vitro and in vivo (18, 19). In vivo, the major source of IL-6 within the bone marrow is the compartment of adherent cells, especially stromal cells (19, 46, 47). Most data have indicated that myeloma cell growth was IL-6 paracrine rather than autocrine, making an autocrine CD40-induced IL-6 growth of myeloma cells unlikely in vivo. Indeed, the expression of CD40L has been thus far described essentially on T lymphocytes, exclusively on activated T cells, that are not found particularly within the bone marrow in contact with the myeloma cells. Moreover, autologous CD3-activated T lymphocytes have been shown to reduce myeloma cell proliferation through cell to cell contacts: this study did not specify whether CD40-CD40L interactions were involved but CD40L was probably expressed on the CD3-activated T cells (48).

The role of CD40 on normal plasma cells, if any, remains unknown. Recent data have shown that CD40 stimulation of germinal center B cells allowed the generation of memory B cells without differentiation into plasma cells, and that terminal differentiation of germinal center B cells into plasma cells could only be obtained upon removal of CD40 stimulation (49). On the other hand, tonsillar plasma cells were rescued from apoptosis by bone marrow fibroblasts but not upon CD40 stimulation (50). These results indicate that triggering CD40 does not allow any proliferation or any survival of normal plasma cells. The mechanism by which normal plasma cells survive in the bone marrow for a certain time period before dying remains to be explained, but does not seem to involve CD40.

In this report, we have demonstrated that CD40 triggering on the XG2 myeloma cell line induced a cell growth arrest mediated by LFA-1 and one of its ligand, ICAM-2. Of interest, we have previously shown that CD40 was expressed on all normal plasma cells and on the broad majority of malignant plasma cells. On malignant plasma cells, we have demonstrated that CD40 was expressed throughout the different phases of disease progression, while LFA-1 expression was dramatically reduced during disease acceleration (16, 37). Taken together, our data emphasize the potential involvement of LFA-1 in the control of MM progression and raise the perspective of a new field of investigations.

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REFERENCES


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