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Differential Use of Very Late Antigen-4 and -5 Integrins by Hematopoietic Precursors and Myeloma Cells to Adhere to Transforming Growth Factor-β1-treated Bone Marrow Stroma*

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The very late antigen (VLA)-4 and VLA-5 integrins mediate hematopoietic progenitor cell attachment to bone marrow (BM) stroma. Transforming growth factor-β1 (TGF-β1) is a cytokine present in the BM microenvironment that has been shown to regulate the synthesis of adhesion elements in several cell types. We have investigated whether TGF-β1 action on human BM stromal cells affected the adhesion of progenitor cells involving integrins VLA-4 and VLA-5. Two precursor cell lines, pre-B Nalm-6 and the multipotential UT-7, attached to untreated primary stroma and to the human BM stromal cell line Str-5 preferentially using VLA-4. However, treatment of the stroma with TGF-β1 resulted in a significant reduction in the participation of VLA-4 in mediating precursor cell adhesion to stroma and a concomitant increase in the utilization of VLA-5. This effect was not exclusive of normal BM stroma. Treatment with TGF-β1 of stroma from multiple myeloma BM samples produced a substantial increase in VLA-5 use by the myeloma cell line NCI-H929 to adhere to this stroma. The differential use of VLA-4 and VLA-5 correlated with an increase in fibronectin surface expression by stromal cells in response to TGF-β1. Adhesion assays to purified fibronectin using Nalm-6 cells showed a predominant utilization of VLA-4 at low concentrations of this ligand, whereas higher concentrations resulted in a preferential use of VLA-5. These results indicate that regulation of fibronectin expression on BM stromal cells by TGF-β1 results in a modulation of the pattern of integrins used by the precursor and myeloma cells to adhere to BM stroma, which could have important consequences on the proliferation and differentiation of hematopoietic precursor cells as well as on the localization and growth of myeloma cells.

The bone marrow stromal cells (BMSC)[1] constitute a population of different cell types that provide the bone marrow (BM) microenvironment with a wealth of cytokines necessary for sustained hematopoiesis and that express membrane ligands for adhesion receptors on hematopoietic progenitor cells (1). The integrins VLA-4 and VLA-5 are among the main adhesion receptors used by $CD34^{high}$, precursor, and leukemic cells to attach to stroma, interacting with different regions on fibronectin (FN), whereas VLA-4 can additionally bind to VCAM-1, which is constitutively expressed by BMSC (2–5).

TGF-β is a multifunctional cytokine present in the BM microenvironment that is mainly produced by megakaryocytes and by BMSC (6, 7) and that regulates stem cell proliferation and B lymphopoiesis (8–10). The levels of TGF-β have been found to be higher in several leukemias, such as multiple myeloma and B cell chronic lymphocytic leukemia, compared with normal bone marrow (11, 12). We have recently characterized the TGF-β1 receptor system on primary human BMSC, showing the expression of the serine/threonine kinase transducing type I and II receptors as well as endoglin (13). Little is known about the effects of TGF-β on stromal cell function. Potential targets for its action include the control of cell proliferation and the regulation of the synthesis of adhesion elements, such as extracellular matrix proteins, and extracellular matrix protein-degrading proteases (14). Changes in the expression of adhesion elements on BMSC at different niches or during different stages of differentiation could result in variations in the adhesion of maturing precursor cells and could also influence the localization of tumor cells in the BM. In this work, we have studied the involvement of integrins VLA-4 and VLA-5 in progenitor cell adhesion to TGF-β1-treated stroma.

MATERIALS AND METHODS

Stromal Cell Cultures—Normal BM was obtained after informed consent from donors for allogeneic bone marrow transplants. Multiple myeloma BM samples were obtained from untreated patients with active disease. The stromal cultures were generated as described previously (13) and maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum (complete medium) and 10^{-5} M hydrocortisone sodium succinate. Non-adherent cells were removed after 1 week, and upon confluency, the stromal cells were passaged by trypsin/EDTA. After four passages, they were seeded 1 week before use in 96-well plates for adhesion and cell ELISAs. Our BM stromal monolayers consisted mainly of fibroblasts and macrophages, with a low percentage of endothelial cells (13). The human bone marrow stromal cell line Str-5 was maintained in complete medium (13). For TGF-β1 treatments, stromal cultures were first incubated in IMDM supplemented with 1% fetal calf serum and serum replacement medium (2× TCM, ICN Biomedicals) for 24 h, followed by a 24-h incubation in the same medium in the presence of recombinant human TGF-β1 (R&D Systems, Abingdon, United Kingdom), and this medium was removed by aspiration before assays.

Cells and Antibodies—The pre-B Nalm-6, myeloma NCI-H929, and Burkitt lymphoma Ramos cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (and 50 μM 2-mercaptoethanol for the NCI-H929 cells). The multipotential cell line UT-7 (15), which expresses CD34 and CD33, was grown in α-minimum Eagle's medium in the presence of 10% fetal calf serum and 2 ng/ml granulocyte-macrophage colony-stimulating factor. Antibodies used in this study included monoclonal P3X63 (16), anti-α4 HP1/2 (17), anti-α5 SAM-1 (18), and P1D6 (Life Technologies, Inc.), Bear-1 anti-CD11b (18),

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[1] The abbreviations used are: BMSC, bone marrow stromal cells; BM, bone marrow; VLA, very late antigen; FN, fibronectin; VCAM-1, vascular cell adhesion molecule-1; TGF-β, transforming growth factor-β; IMDM, Iscove's modified Dulbecco's medium; ELISA, enzyme-linked immunosorbent assay; BCECF-AM, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester; mAb, monoclonal antibody.

anti-VCAM-1 4B9 (19), and polyclonal anti-FN central cell-binding domain Rb110 and anti-FN carboxyl-terminal CS-1-containing cell-binding domain Rb113.

Measurement of Bioactive TGF-β—TGF-β levels from stromal supernatants were determined in a bioassay by analyzing the growth inhibition of mink lung epithelial Mv1Lu cells (American Type Culture Collection CCL 64). Mv1Lu cells were subcultured in IMDM supplemented with 10% fetal calf serum in 24-well tissue culture plates at 3×10^4 cells/well. TGF-β1 (0.1–10 ng/ml) or 24-h stromal supernatants in IMDM supplemented with 1% fetal calf serum and 2× TCM were added without previous acidification to Mv1Lu cells in triplicates, which were incubated for 24 h at 37 °C, the last 4 h in the presence of 1 μCi/well [^{3}H]thymidine (Amersham International, Buckinghamshire, UK). At the end of incubation, cells were washed with phosphate-buffered saline and extracted with 0.2 N NaOH, and extracts were neutralized with 1 M HCl before adding scintillation liquid and analyzed in a β-counter.

Adhesion Assays—Cell lines were labeled in their respective growth medium with the fluorescent dye BCECF-AM (Molecular Probes, Leiden, The Netherlands) and finally resuspended in RPMI 1640 medium containing 0.4% bovine serum albumin (adhesion medium). For cell adhesion to purified plasma fibronectin (Sigma), increasing concentrations of this ligand were coated overnight at 4 °C in 100 mM $NaHCO_3$ (pH 8.8), followed by 2-h incubation at 37 °C and then blocking with 0.4% bovine serum albumin in the same solution for 2 h at 37 °C. Preincubations with antibodies were carried out at 37 °C for 15 min, and the antibodies were removed before adhesion. To untreated or TGF-β1-treated stroma or fibronectin in 96-well dishes (Falcon) was added 50 μl of adhesion medium 20 min before adhesion, and after removing it, labeled cells (5×10^4 cells/well) were incubated with the stroma or with fibronectin for 20 min at 37 °C. Unbound cells were removed by three washes with RPMI 1640 medium, and adherent cells were quantified using a fluorescence analyzer (CytoFluor 2300, Millipore Corp.).

Cellular ELISA—Untreated or TGF-β1-treated stromal monolayers were fixed with 3.7% formaldehyde in phosphate-buffered saline, washed three times with Tris-buffered saline, and blocked with 3% bovine serum albumin in Tris-buffered saline. After removing the blocking solution, cells were incubated for 1 h at 22 °C with the primary antibodies, washed with Tris-buffered saline in the presence of 0.05% Tween 20, and further incubated for 1 h at 22 °C with peroxidase-conjugated goat anti-rabbit Ig (Dako, Glostrup, Denmark). Monolayers were finally washed and incubated with ABTS (Boehringer Mannheim) in the dark before measuring in an ELISA reader (Multiskan Bichromatic, Labsystems, Helsinki, Finland) at 405 nm.

Flow Cytometry—Preparation of the stromal cells for flow cytometry analysis was performed as described previously (13). Approximately 10^5 stromal cells were incubated with saturating concentrations of primary antibodies at 4 °C for 45 min. After washing, fluorescein isothiocyanate-conjugated rabbit anti-mouse Ig (Dako) was added and further incubated at 4 °C for 30 min. Samples were analyzed using a Coulter Epics XL flow cytometer.

RESULTS

To investigate whether TGF-β1 action on BMSC could influence hematopoietic precursor and myeloma cell adhesion mediated by integrins VLA-4 and VLA-5, we first measured the levels of bioactive TGF-β present in primary human BMSC (HBM-Str) cultures, in the stromal cell line Str-5, and in multiple myeloma BMSC (MM-Str) cultures. Bioassays for TGF-β activity using the TGF-β responder cell line Mv1Lu showed TGF-β-dependent activity corresponding to 0.3–0.6 ng/ml in HBM-Str cells cultured for 24 h, whereas Str-5 and MM-Str cells secreted bioactive TGF-β in the range of 2–2.5 ng/ml (Fig. 1).

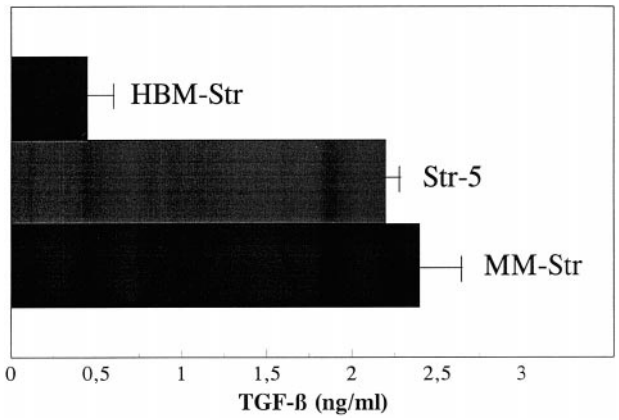

FIG. 1. **Secretion of bioactive TGF-β by BMSC.** Stromal cultures from healthy donors (n = 4; HBM-Str), the stromal cell line Str-5 (n = 4), and multiple myeloma BM samples (n = 4; MM-Str) were incubated for 24 h in IMDM, 1% fetal calf serum, and 2× TCM, and the supernatants were analyzed for their TGF-β content in a bioassay using Mv1Lu cells. Data represent the mean ± S.D. of triplicate samples from a representative experiment.

Incubation of primary stroma with 1 ng/ml TGF-β1 resulted in a decrease in the adhesion of the pre-B cell line Nalm-6 compared with the adhesion to untreated stroma (Fig. 2*A*). The anti-α4 HP1/2 mAb significantly inhibited (30–40%; $p < 0.05$) the adhesion of Nalm-6 cells to untreated stroma, but inhibition by HP1/2 was consistently decreased when the adhesion was performed on TGF-β1-incubated stroma. In contrast, the anti-α5 SAM-1 mAb minimally inhibited Nalm-6 cell adhesion to untreated stroma; however, incubation of stroma with TGF-β1 resulted in a blockade of cell adhesion by SAM-1, as well as by another anti-α5 mAb called P1D6 (data not shown), of ~50% ($p < 0.05$) (Fig. 2*A*). The results of inhibition of Nalm-6 cell adhesion using a mixture of anti-α4 and anti-α5 mAbs mimicked those of inhibition with single mAbs. Thus, HP1/2 and SAM-1 together inhibited slightly better than HP1/2 alone the adhesion of Nalm-6 cells to untreated stroma (Fig. 2*A*), reflecting a major VLA-4 use. After incubation of the stroma with TGF-β1, the combination of both antibodies inhibited similarly to SAM-1 alone, indicating a predominant VLA-5 utilization. The anti-VCAM-1 4B9 mAb inhibited by 15–20% the adhesion of Nalm-6 cells to either untreated or TGF-β1-treated stroma (Fig. 2*A*). The effect of TGF-β1 action on Nalm-6 cell adhesion to HBM-Str was also evident when we preincubated the Str-5 cells with increasing concentrations of TGF-β1, although in this case, higher concentrations of the cytokine were needed to obtain a substantial blocking of Nalm-6 cell adhesion by the anti-α5 mAb (Fig. 2*B*). In parallel experiments, the SAM-1 and P1D6 mAbs did not inhibit the adhesion of the VLA-5-negative B cell line Ramos either to untreated or TGF-β1-treated HBM-Str cells (data not shown).

As for the case of Nalm-6 cells, preincubation of HBM-Str cells with TGF-β1 resulted in a decrease in the adhesion of the multipotential cell line UT-7 (Fig. 2*C*). Interestingly, both HP1/2 and, to a lesser extent, SAM-1 inhibited the adhesion of UT-7 cells to untreated stroma. However, TGF-β1 treatment of the stroma resulted in a notable reduction in HP1/2 inhibition of cell adhesion, whereas inhibition by SAM-1 remained unchanged, indicating a predominant VLA-5 use. We could not observe any clear effect of the anti-VCAM-1 mAb on UT-7 cell adhesion to stroma, suggesting that VLA-4 on these cells mainly interacts with fibronectin.

To study if the differential use of VLA-4 and VLA-5 could also be observed using leukemic BM stroma, we generated stromal cell cultures from multiple myeloma BM samples and analyzed the effect of their incubation with TGF-β1 on the adhesion of the myeloma-derived cell line NCI-H929. These monolayers expressed abundant fibronectin and substantial levels of VCAM-1 (data not shown).[2] Similarly to Nalm-6 and UT-7 cells, NCI-H929 cells adhered significantly less to TGF-β1-treated compared with untreated multiple myeloma stroma (Fig. 3). The anti-α4 HP1/2 mAb inhibited NCI-H929 cell adhesion to untreated stroma by ~40% ($p < 0.05$) and to a lower

[2] M. M. Robledo, F. Sanz-Rodriguez, A. Hidalgo, and J. Teixidó, manuscript in preparation.

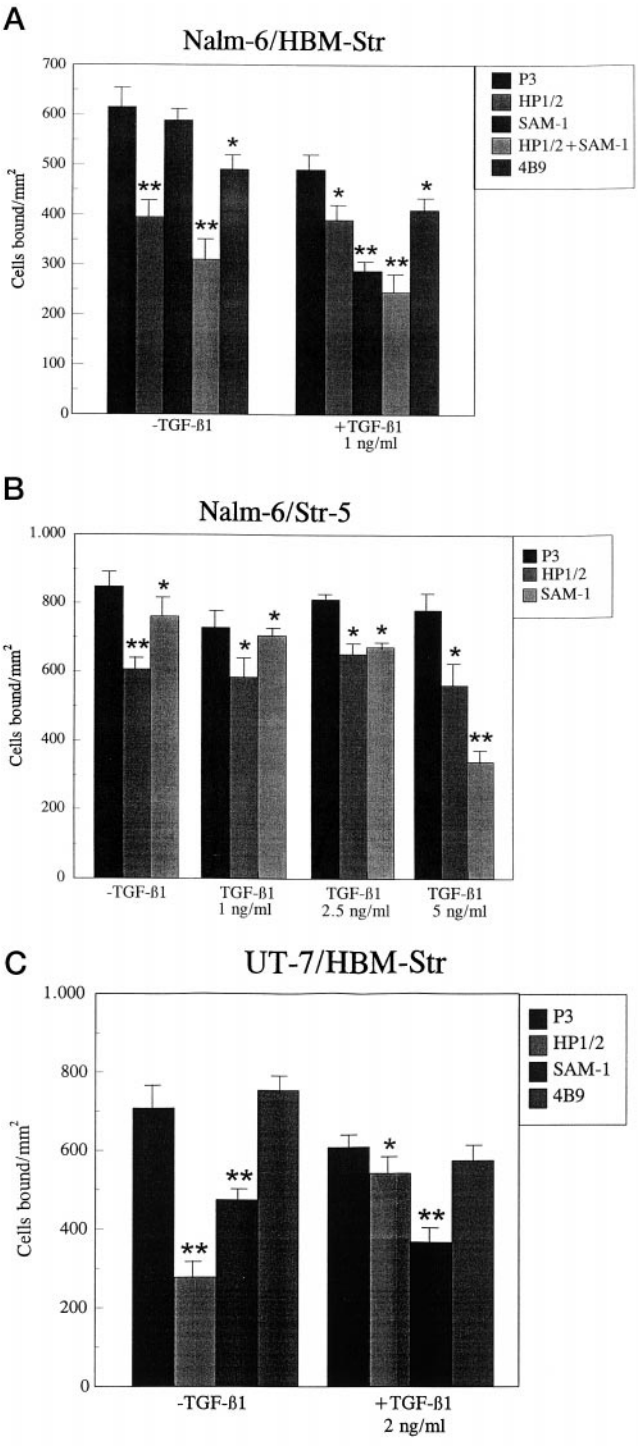

FIG. 2. **Effect of TGF-β1 treatment of human BMSC on VLA-4- and VLA-5-dependent Nalm-6 and UT-7 cell adhesion.** HBM-Str (*A* ($n = 4$) and *C* ($n = 3$)) and Str-5 (*B* ($n = 4$)) monolayers were incubated for 24 h in the absence or presence of the indicated concentrations of TGF-β1, which was removed before adhesion. BCECF-AM-labeled Nalm-6 and UT-7 cells were preincubated for 15 min at 37 °C with optimal concentrations of anti-α4 HP1/2, anti-α5 SAM-1, anti-VCAM-1 4B9, or control P3 mAb before the adhesion assay. Adhesion was quantified in a fluorescence analyzer. **, adhesion was significantly inhibited ($p < 0.05$) according to Student's two-tailed *t* test; *, inhibition was observed, but was not quite low enough to meet the $p < 0.05$ confidence level.

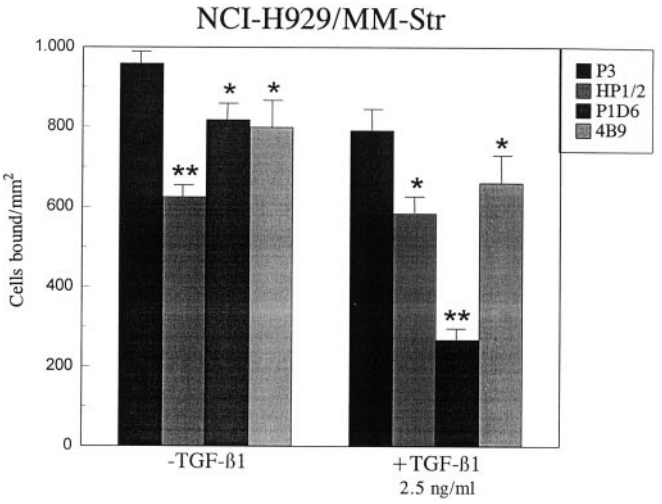

FIG. 3. **Effect of TGF-β1 treatment of multiple myeloma stroma on VLA-4- and VLA-5-dependent myeloma NCI-H929 cell adhesion.** Multiple myeloma stromal cells were incubated for 24 h in the absence or presence of TGF-β1, and the adhesion of NCI-H929 cells ($n = 3$), previously incubated with the indicated mAb, was quantified with a fluorescence analyzer. **, adhesion was significantly inhibited ($p < 0.05$) according to Student's two-tailed *t* test; *, inhibition was observed, but was not quite low enough to meet the $p < 0.05$ confidence level.

extent after treatment with TGF-β1. As observed for Nalm-6 cells, a low degree of inhibition of NCI-H929 cell adhesion to untreated stroma was detected with the anti-α5 mAb P1D6, but after TGF-β1 incubation of the multiple myeloma stroma, a high induction of blocking by this antibody was obtained (Fig. 3). The anti-VCAM-1 4B9 mAb inhibited normally 15–25% of NCI-H929 cell adhesion to both untreated and TGF-β1-treated multiple myeloma stroma.

One of the targets of TGF-β action is the control of extracellular matrix protein synthesis (14, 20). As fibronectin is expressed by stromal cells and is a ligand for both VLA-4 and VLA-5, we analyzed whether FN levels on the surface of stromal cells were affected by TGF-β1. Cellular ELISAs consistently showed an augmentation in FN deposition by HBM-Str cells in response to TGF-β1, as measured by antibodies against the FN central cell-binding domain and the CS-1-containing region (Fig. 4). These results were confirmed by [^{35}S]Met/Cys metabolic labeling of stroma, where a 2.5-fold increase in gelatin-bound extracellular FN was observed upon TGF-β1 treatment (Fig. 4). When the expression of VCAM-1 on stromal cells was analyzed by flow cytometry, a significant reduction was obtained in response to TGF-β1 (data not shown), similar to previous results (21). The same experiments showed no effect of TGF-β1 on VLA-β1 or CD11b expression on these cells.

To investigate possible mechanisms underlying the potential preferential receptor usage, we carried out cell adhesion assays with increasing concentrations of human plasma fibronectin using Nalm-6 cells. The cells adhered to fibronectin in a concentration-dependent manner in the presence of control P3 antibodies, reaching saturation at ~30 μg/ml (Fig. 5). The inhibition of Nalm-6 cell adhesion to fibronectin by anti-α4 and anti-α5 antibodies displayed three different patterns, depending on the concentrations of fibronectin used. At the lowest concentrations of FN (2–4 μg/ml), the anti-α4 HP1/2 mAb notably inhibited Nalm-6 cell adhesion, whereas the anti-α5 P1D6 mAb did not block, suggesting a predominant VLA-4 use (Fig. 5). When the concentration of FN was increased (8–12 μg/ml), HP1/2 showed no or modest inhibition of Nalm-6 cell adhesion, whereas P1D6 significantly inhibited this adhesion, indicating a preferential VLA-5 utilization. At the highest concentrations of fibronectin (>16 μg/ml), both P1D6 and HP1/2 inhibited Nalm-6 cell adhesion, although the former inhibited always to a higher extent (Fig. 5). In the same experiments, a mixture of antibodies against the FN central cell-binding do-

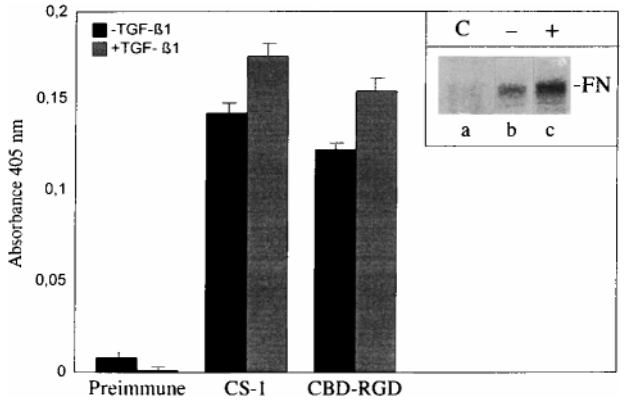

FIG. 4. **Effect of TGF-β1 treatment of human BMSC on fibronectin expression.** HBM-Str cells were incubated for 24 h with 2 ng/ml TGF-β1, and the expression of the central cell-binding domain (*CBD-RGD*) and the carboxyl-terminal CS-1 region of FN was analyzed by cellular ELISA using polyclonal antibodies Rb110 and Rb113, respectively. *Inset*, stromal cells that had been incubated for 24 h in the absence (−) or presence (+) of TGF-β1 were metabolically labeled for 3 h with [^{35}S]Met/Cys (Amersham Pharmacia Biotech) maintaining the cytokine, and extracellular matrix proteins were extracted in a urea-containing buffer as described (20). Extracts were incubated overnight at 4 °C either with Sepharose CL-4B (*lane a*) or with gelatin-Sepharose CL-4B (*lanes b* and *c*). Complexes were washed, and FN was released by resuspending the beads in electrophoresis sample buffer and heating at 100 °C for 2 min. Electrophoresis was carried out using 5% polyacrylamide gels, and bands were visualized with a PhosphorImager.

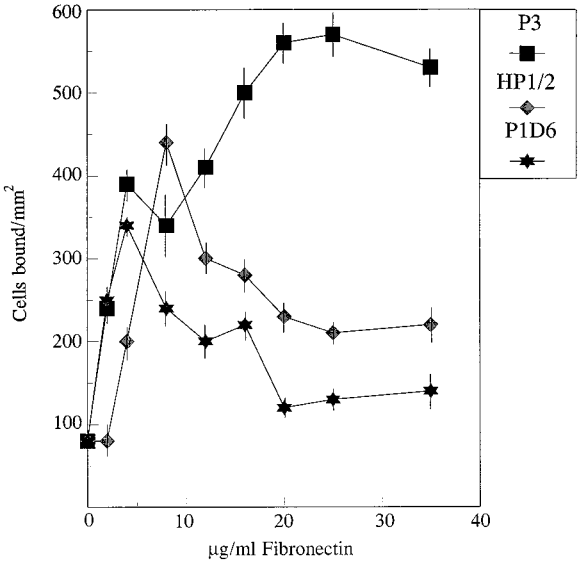

FIG. 5. **Effect of anti-α4 and anti-α5 antibodies on adhesion of Nalm-6 cells to fibronectin.** BCECF-AM-labeled Nalm-6 cells were incubated with saturating concentrations of anti-α4 HP1/2, anti-α5 P1D6, or control P3 mAb, and after removing the antibodies by centrifugation, cells were added to wells coated with increasing concentrations of fibronectin and incubated for 20 min at 37 °C. Adhesion was quantified in a fluorescence analyzer. Data represent the mean ± S.D. of triplicate samples from a representative result of five separate experiments.

main and the CS-1-containing region fully inhibited Nalm-6 cell adhesion when they were preincubated with the fibronectin-coated wells (data not shown), demonstrating the specificity of the adhesion.

DISCUSSION

This study describes novel modulatory roles for TGF-β1 in the adhesion of hematopoietic precursor and myeloma cells to BM stroma. The important finding is that TGF-β1 action on human BMSC resulted in a significant reduction in the participation of VLA-4 in mediating precursor and myeloma cell adhesion to the stroma, linked to an increase in VLA-5 involvement in such adhesion. As shown in Table I, the ratio of VLA-5 *versus* VLA-4 utilization by Nalm-6 and UT-7 cells increased 10-fold after treatment of the stroma with TGF-β1, whereas this ratio was close to 5-fold in the case of NCI-H929 cells, as determined from values of inhibition of cell adhesion. The results were obtained using TGF-β1 concentrations similar to those found in the supernatants from normal and multiple myeloma stromata, although in UT-7 cells, slightly higher amounts of TGF-β1 were required. We detected lower levels of TGF-β in multiple myeloma stromal cultures compared with a previous report (11), which could be due to the different methods of measurement, as we analyzed bioactive TGF-β, whereas the other study used an ELISA. The differential effects on VLA-4- and VLA-5-mediated cell adhesion are likely at the attachment and possibly spreading levels, as we performed short adhesion assays (20 min), and thus, little, if any, cell migration took place in our system. In addition, it has been reported that most of VLA-4-mediated B cell precursor adhesion to stroma occurs in the first 15 min of cell attachment (22), and therefore, our results were obtained under conditions favoring VLA-4 activity. The modulation of VLA-4 and VLA-5 use by TGF-β1 treatment of stroma takes place in the absence of any exogenously added TGF-β1 in the precursor cell lines, and thus, it is independent of a possible increase in VLA-5 expression due to this cytokine, as has been reported for several cell types (23, 24).

TABLE I

Effect of treatment of BM stroma with TGF-β1 on the differential use of VLA-4 and VLA-5 by Nalm-6, UT-7, and NCI-H929 cells

Shown are the ratios corresponding to values of inhibition of cell adhesion obtained with anti-α5 and anti-α4 mAbs from the experiments shown in Figs. 2 and 3.

Cell line	α5/α4 ratio inhibition		−/+TGF-β1 ratio
	−TGF-β1	+TGF-β1	
Nalm-6	0.14	1.40	10
UT-7	0.52	5.25	10
NCI-H929	0.46	2.16	4.7

Associated with the decreased implication of VLA-4 in mediating precursor and myeloma cell adhesion to TGF-β1-treated stroma, there was a reduction in adhesion to stroma. A diminished B cell precursor cell adhesion to stroma preincubated with TGF-β1 was also previously reported, which was linked to a reduced expression of VCAM-1 (21). In the present work, we observed a moderate participation of the VLA-4/VCAM-1 adhesion pathway in Nalm-6 and NCI-H929 cells, as detected by antibody inhibition of the adhesion, but this interaction was not reduced upon stroma treatment with TGF-β1, suggesting that other adhesion pathways might be affected. This suggests that most of the VLA-4-dependent precursor and myeloma cell adhesion to untreated stroma that we observed takes place by attachment to fibronectin. The data also indicate that the increase in VLA-5 involvement in cell adhesion to TGF-β1-treated stroma does not restore the reduced adhesion.

The binding of TGF-β1 to the stroma resulted in an increase in the levels of fibronectin expressed on the surface of stromal cells. Although both the CS-1 and central cell-binding domains of FN were augmented by TGF-β1, the precursor and myeloma cells used predominantly VLA-5 to interact with FN on TGF-β1-treated stroma. To gain some insight into the mechanisms leading to the preferential use of VLA-5 after increased fibronectin deposition on BMSC by TGF-β1, we tested whether changes in the concentration of purified fibronectin could influence the integrins utilized to mediate cell adhesion. The results obtained in the lower and likely more physiological

range of FN concentrations mimicked those from adhesion to TGF-β1-treated stroma. Thus, VLA-4 was the predominant receptor used at the lowest concentrations of FN inside this range, whereas VLA-5 was the preferential integrin utilized when the concentration of FN was increased. At high concentrations of fibronectin, VLA-5 use was still higher than VLA-4 use, although the latter retained considerable participation in adhesion to fibronectin. These results suggest that the concentrations of fibronectin modulate the receptor used to mediate cell adhesion, and therefore, TGF-β1 regulation of the expression levels of fibronectin on BMSC finely tunes the pattern of the integrins that will be used by the precursor and myeloma cells to interact with the stroma.

The α4 integrins are required for normal development of both B and T precursor cells in bone marrow (25), and long-term BM cultures have demonstrated the involvement of VLA-4 in lymphopoiesis (26), evidencing the importance of VLA-4-mediated adhesion during hematopoiesis. It is well established that VLA-4 and VLA-5 on stem and precursor cells as well as on several leukemic cells mediate attachment to their ligands on BMSC (2–5). This interaction is important for the differentiation and proliferation of hematopoietic progenitor cells. For instance, VLA-4/FN interaction is required for terminal maturation of Ig-secreting BM cells (27). In addition, VLA-4- and VLA-5-mediated cell adhesion to FN decreases the proliferation of both normal hematopoietic and chronic myelogenous leukemia progenitors (28, 29), and it has been reported that VLA-5/FN interaction induces apoptosis of hematopoietic progenitor cells (30). TGF-β is present in the BM microenvironment, and it is conceivable that changing amounts of this cytokine in different niches of BM could influence the regulation of hematopoiesis. BMSC secrete TGF-β1 and have the TGF-β1 receptor system necessary for signaling by this cytokine (13). The differential use of VLA-4 and VLA-5 by progenitor and myeloma cells to adhere to stroma after binding of TGF-β1 to their receptors on the stromal cells suggests a mechanism by which changing adhesive interactions might lead to a modulation of hematopoietic progenitor cell proliferation and differentiation. Additionally, the differential contribution of VLA-4 and VLA-5 to the mediation of myeloma cell attachment to multiple myeloma stroma could influence the localization and proliferation of the malignant plasma cells in bone marrow.

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Differential Use of Very Late Antigen-4 and -5 Integrins by Hematopoietic Precursors and Myeloma Cells to Adhere to Transforming Growth Factor- β 1-treated Bone Marrow Stroma

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