 Constitutive Up-Regulation of Integrin-mediated Adhesion of Tumor-infiltrating Lymphocytes to Osteoblasts and Bone Marrow-derived Stromal Cells

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ABSTRACT

Tumor-reactive T cells, known as tumor-infiltrating lymphocytes (TILs), are known to infiltrate various tumors. Although TILs exert cytotoxic activities against tumor cells, only a small percentage of tumors usually contain TILs that specifically react to tumor antigens. Because the exact role of these lymphocytes is unclear, we investigated the mechanisms of migration and adhesion of TILs to bone metastatic tumors, particularly to osteoblasts and bone marrow-derived stromal cell (BMSC). Histopathological examination showed that most TILs in secondary bone metastatic tumors (from primary tumors in the lung or breast) were found in the supporting tissue stroma between the bone and tumor mass. Cultured TILs (obtained from breast tumors) adhered spontaneously to osteoblasts and BMSCs (obtained from patients with osteoarthritis) without exogenous stimulation. Adhesion was further enhanced by chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β. TILs highly expressed activation antigens CD25 and CD69. A spontaneous activation of an integrin, lymphocyte function-associated antigen-1 (LFA-1), was also detected on TILs. TILs produced high concentrations of MIP-1α and MIP-1β and spontaneous polymerization of cytoskeletal F-actin was observed in these cells. Adhesion of TILs to osteoblasts and BMSCs via LFA-1 and very late antigen-4 was associated with the production of osteoclastogenic interleukin 6 by the latter cells. Our results indicate that integrins on TILs are activated in an autocrine manner by MIP-1α and MIP-1β, and that treatment with the chemokines increases the binding of TILs on osteoblasts and stromal cells via a mechanism involving intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 as targets for the integrin. Our data also indicated that interactions between TILs and osteoblasts/stromal cells lead to the secretion of the latter of the osteoimmunocyte cytokine interleukin 6.

INTRODUCTION

Tumor-reactive T cells mediate tumor regression as evident by clinical response to adoptive immunotherapy with TILs isolated from a variety of tumors such as melanomas (1, 2). The efficacy of cancer immunotherapy using cultured TILs depends on the migration of infused TILs into tumor-bearing tissue and encountering tumor cells to mediate anti-tumor responses. Integrins on TILs play a pivotal role in the adhesion of TILs to both endothelial cells and tumor cells during TILs migration into the neoplastic tissue. Two integrins, LFA-1 and VLA-4, on T cells are known to mediate T-cell adhesion to their ligands on endothelial cells as well as tumor cells. The adhesive capacity of integrins is tightly regulated. Although integrins that are expressed on resting cells do not mediate a firm adhesion of these cells to their ligands, stimulation of these cells results in a rapid increase in integrin function (3–5). During such activation, a signal transduced to lymphocytes converts the functionally inactive integrin to an active adhesive configuration. In this regard, we have reported previously (6–8) that the chemokine MIP-1β triggers integrin and induces adhesion of T-cell subsets to endothelial integrin-ligands. Several recent studies have supported the potential importance of chemokines in inflammatory responses. Thus, various chemokines, including MIP-1β, that are produced in large amounts at the site of inflammation activate integrins on leukocytes and result in a relative leukocytosis in the tissues. The mechanisms that cause activation of integrin are thought to involve conformational changes in the ectodomain of integrins and/or clustering of integrins on the cell membrane, resulting from cytoskeletal actin-polymerization associated with the endodomain of integrins (9–11). However, the exact mechanisms that enhance the adherence of TILs to their target cells or the mechanisms that regulate integrin adhesiveness to targets are not very clear at present.

Although TILs induce anti-tumor responses in certain neoplasms, they proliferate poorly in tumor tissues, and only a small percentage of tumors contain TILs that specifically react to tumor antigens (12). Moreover, certain TILs proliferate on stimulation with autologous tumors, but most TILs do not develop cytotoxic activity against either autologous or heterologous targets (13, 14). Thus, the exact role of these lymphocytes is unclear. It is possible that lymphocytes that accumulate around or within tumors may respond to those antigens that are expressed in response to tissue injury and repair and may function in a manner similar to lymphocytes at the site of chronic inflammation. T cells migrate into inflamed tissue and are known to play a major role in a self-defense mechanism. However, in chronic inflammation, accumulation of T cells sometimes results in tissue destruction, which may lead to the development of chronic diseases. Thus, it can be assumed that TILs that migrate to neoplastic tissues to attack tumor cells also play a negative role by inducing local tissue damage.

Various tumor cells often metastasize into the bone, which results in unregulated bone resorption and subsequent hypercalcemia (15–18). Multiple local soluble factors and systemic hormones closely regulate the two major processes of bone remodeling, bone formation and resorption. Cellular interactions involved in the development of bone cells, however, seem to be also mediated by cellular adhesion. We have reported recently (19) that osteoblasts express adhesion molecules, ICAM-1 and VCAM-1, and that cellular adhesion through these molecules to immune cells induces the production of bone-resorbing cytokines from osteoblasts. It is well known that severe infiltration of lymphocytes occurs around solid bone metastatic tumors as well as in normal cancer tissue. It is possible that tumor cells may adhere to osteoblasts and modulate bone metabolism. However, the majority of tumor cells derived from solid tumors including lung, breast, prostate, and colon cancer do not express LFA-1/VLA-4.

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3 The abbreviations used are: TIL, tumor-infiltrating lymphocyte; BMSC, bone marrow-derived stromal cell; ICAM-1, intercellular adhesion molecule-1; LFA-1, lymphocyte function-associated antigen-1; MFI, mean fluorescence intensity; MIP-1, macrophage inflammatory protein-1; PBMC, peripheral blood mononuclear cell; PBT, peripheral blood T cell; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4; Ab, antibody; mAb, monoclonal Ab; PMA, phorbol 12-myristate 13-acetate; IL, interleukin.
which are suitable adhesion receptors to ICAM-1/VCAM-1 on osteoblasts, which indicates that these cells cannot adhere to osteoblasts. The relationship between bone metabolism and the immune system is a subject of great interest at present (20), and it is possible that TILs may affect osteoblasts and subsequently bone metabolism in patients with secondary bone tumors.

On the basis of this background, we investigated in the present study the expression and chemokine-mediated regulation of integrins on TILs obtained from patients with breast or lung cancer. We also investigated the importance of the adhesive features of TILs on bone metabolism and induction of production of osteoclastogenic cytokine, IL-6, by examining the molecular mechanisms of their adhesion to osteoblasts or BMSCs.

MATERIALS AND METHODS

Purification of Human Peripheral T Cells and TILs. The study design was approved by the Human Ethics Review Committees of the participating institutions. Tumors from 12 patients with lung or breast cancer were surgically removed for therapeutic purposes. Tissue samples from secondary bone tumors originating from breast cancers were snap-frozen for histological examination. PBMCs were isolated from peripheral blood of normal volunteers by Ficoll-Hypaque density-gradient centrifugation (LSM, Organon Teknika, Durham, NC). TILs were prepared from lung and breast cancer as described previously (21). Briefly, after excision, the fresh tumor tissue was cut into small pieces with scissors, then placed in a flask containing a mixture of 0.1 mg/ml DNase type I, 1 mg/ml collagenase type IV, and 0.5 mg/ml hyaluronidase type V (all from Sigma-Aldrich Japan, Tokyo) in RPMI 1640. The mixture was stirred at room temperature for 2 to 4 h. The resultant cell suspension was washed in HBSS (Nissui, Tokyo) and subjected to LSM centrifugation. The CD3-positive T cells were negatively selected as previously described (5). Briefly, the PBMC or tissue samples obtained using LSM were incubated with a cocktail of mAbs [consisting of CD19 mAb FMC63 (Dr. H. Zola, Child Health Research Institute, North Adelaide, Australia), CD16 mAb 3G8 (Dr. D. Siegel, National Institutes of Health, Bethesda, MD), CD11b mAb NIH11b-1 (Dr. S. Shaw, National Institutes of Health, Bethesda, MD), and CD14 mAb 63D3 (American Type Culture Collection, Rockville, MD)] and with antirat IgG Ab for 30 min at 4°C, which also allowed mAb binding, the plates were rapidly warmed to 37°C for 30 min, then gently washed twice with RPMI 1640 at room temperature to completely remove nonadherent PBTS or TILs. The contents of each well containing adherent PBTS or TILs were lysed with 250 μl of 1% Triton X-100 (Sigma), and the emission from each well was measured using a gamma counter. Data were expressed as mean percentage of the binding of test cells from a representative experiment.

Flow Microfluorometry. Staining and flow cytometric analyses of freshly purified human osteoblasts, human BMSCs, normal PBTS, and TILs were performed using a FACScan (Becton Dickinson, Mountain View, CA) and standard procedures as described previously (5, 23). Briefly, 2 × 10^6 cells were incubated with negative control mAb thyl.2, LFA-1 (CD11a) mAb TS1/22, (American Type Culture Collection), VLA-4 (CD49d) mAb NIH49d-1, ICAM-1 (CD54) mAb 84H10 (S. Shaw), activated LFA-1 mAb NKL-L16, CD69 mAb FN50, CD25 mAb M-A251 (Fujisawa, Osaka, Japan), or VCAM-1 (CD106) mAb 2G7 (Dr. W. Newman, Osaka, Japan). Staining of T cells with mAbs was detected by gating on CD4+ or CD8+ cells using FACSScan. Amplification of the mAb-binding was provided by a three-decade logarithmic amplifier. Quantification of cell surface antigens on single cell was calculated using standard beads (QIKKIT, DAKO Japan, Kyoto, Japan).

F-actin Polymerization Assay. For microscopic analysis, normal PBTS, PMA-activated PBTS, or TILs were allowed to settle for 30 min at 4°C on fibronectin-coated slides. After incubation for 1 min at 37°C, the cells were fixed with 1% formaldehyde. F-actin was stained with rhodamine-phalloidin (1 unit/slide, Molecular Probes, Inc., Eugene, OR) and analyzed later with a confocal laser microscope system (model LSM 410UV, LD Achroplan 20 objective lens, Carl Zeiss, Germany).

ELISA. PBTS from normal subjects and TILs (1 × 10^6) from patients were washed in PBS and lysed with 250 μl of PBS containing 2% N-ocetyl-β-D-glucopyranoide (Sigma-Aldrich, Japan). The culture supernatants were collected from PBTS or TILs (1 × 10^6) after incubation for 24 h in RPMI 1640 with 5% FCS at 37°C without any stimulation. The concentration of MIP-1α and MIP-1β protein in each sample was measured by MIP-1α and MIP-1β ELISA system (R&D Systems, Minneapolis, MN). The sensitivity of the assay is 0.6 pg/ml for MIP-1α and 4.0 pg/ml for MIP-1β. The sensitivity was not affected by the presence of N-ocetyl-β-D-glucopyranoide at the concentration used to lyse cells. Results were expressed in ng/ml × 10^6 cells. Osteoblasts or BMSCs were cultured with PMA-activated PBTS or TILs (which were first fixed with formaldehyde to block cytokine production) at 37°C for 24 h in the presence or absence of blocking mAbs, LFA-1 mAb TS1/22 or VLA-4 mAb HP2/1 (F. Sanchez-Madrid, The Princess Hospital, Madrid, Spain) for 30 min at 4°C. After magnetization of the cells twice, nonmagnetized cells were used as purified normal T cells or purified TILs.

Purification of Human Osteoblasts and BMSCs. Osteoblast-like cells were purified from bone explants of metaphysseal trabecular bone in the proximal femur of patients with osteoarthritis during total hip arthroplasty, according to the procedure described previously by Beresford et al. (22). Incubation of dish-adherent cells resulted in a new cellular growth and formation of a confluent monolayer, which contained cells that were more than 95% positive for high alkaline phosphatase activity and were flat polygonal in shape with multiple spinules. Human adult bone marrow mononuclear cells from Ficoll-Hyphaque interfaces were allowed to adhere to flasks, from which adherent stromal cells were propagated in Ex-Cell 300 medium (JRH Biosciences, Lenexa, KS) containing 10% heat-inactivated FCS (JRH Biosciences). Stromal cells were grown to confluence, passaged twice, and then maintained in DMEM (Nissui) with 10% FCS for 3 days and used as BMSCs in the following experiments.

Adhesion Assay. Adhesion assay of normal PBTS or TILs to human osteoblasts or BMSCs was performed as described previously (5, 23). Osteoblast or stromal cells were placed on 48-well culture plates (Costar, Cambridge, MA), coated with 2% gelatin, and cultured to confluence in DMEM containing 10% FCS. The plates were washed three times with PBS before the addition of T cells. Two × 10^5 PBTS or TILs were labeled with 31Cr (Dupont NEN, Wilmington, DE) in RPMI 1640 with 1% human serum albumin and were added to the culture with or without the relevant adhesion-blocking mAb in the presence or absence of PMA (10 ng/ml, Sigma) or a mixture of MIP-1α and MIP-1β (10 ng/ml each). All mAbs were used at a saturating concentration of 10 μg/ml, which was shown in previous studies (5, 23) to produce a maximum inhibition of the relevant adhesive interaction. After a setting period of 30 min at 4°C, which also allowed mAb binding, the plates were rapidly

RESULTS

Adherence of TILs to Osteoblasts and BMSCs. Microscopic examination of tumor tissues invariably shows features of "chronic inflammation," including accumulation of TILs (12). Most TILs in bone metastatic tumors were located in the supporting tissue stroma between the bone and tumor mass rather than in the tumor parenchyma (Fig. 1). The results of histological examination suggested that TILs may interact with not only cancer cells but also other stromal cells such as osteoblasts and BMSCs in the bone/bone marrow. To examine the adhesive properties of TILs, we incubated these cells (obtained from primary tumor tissues of two representative lung cancer patients) with purified human osteoblasts or with BMSCs. TILs spontaneously adhered to osteoblasts and BMSCs after 30 min of incubation without any exogenous stimulus (Fig. 2, A and B). In
contrast, resting PBTs did not adhere to osteoblasts and BMSCs. Adherence of T cells occurred only after they were activated with PMA or a mixture of MIP-1α and MIP-1β, potent stimulators of integrins. Adhesion of TILs to osteoblasts and BMSCs was also augmented after stimulation by PMA or a mixture of MIP-1α and MIP-1β. On the other hand, adhesion of PMA-activated TILs to osteoblasts or BMSCs was inhibited by anti-LFA-1 and or anti-VLA-4 mAbs but not by control anti-MHC class I antigen mAb (Fig. 2, C and D). These results suggest that TILs adhere to osteoblasts or BMSCs via integrins LFA-1 and VLA-4. The results also indicate that integrins on TILs are already activated and that TILs are highly responsive to such stimuli.

Osteoblasts and BMSCs Expressed Both ICAM-1 and VCAM-1. In the next step, we examined the expression of cell surface adhesion molecules on purified osteoblasts and BMSCs. Cells were stained with mAbs to various adhesion molecules and second FITC-labeled antimouse IgG Ab, followed by flow cytometric analysis using FACScan (Fig. 3). Thy1.2 mAb, which specifically binds to mouse T cells, was used as a negative control. The dotted vertical lines represent the cutoff value for a positive staining, inasmuch as more than 99% of the cells that bound with thy1.2 mAb were present within the amplifier. Compared with the negative control, most osteoblasts stained with mAbs to both adhesion molecules, ICAM-1 (>90%) and VCAM-1 (~ 80%; Fig. 3A). BMSCs also expressed both ICAM-1 (80%) and VCAM-1 (70%; Fig. 3B). These two adhesion molecules are members of the immunoglobulin superfamily.

Expression of LFA-1 and Activation Markers CD25 and CD69 on TILs. Using flow cytometry, we next examined whether the enhanced adhesion of TILs to osteoblasts and BMSCs depends on a high expression of adhesion molecules or the expression of activated molecules. Fig. 4 shows the percentage of TILs and PBMCs that stained positive for a variety of mAbs and Fig. 5 shows the MFI for mAb binding, which reflects the quantity of molecules expressed on these cells. The MFI and percentage of LFA-1 positively-stained CD4+ and CD8+ TILs were similar to those of normal CD4+ and CD8+ T cells. Similarly results were observed for VLA-4. In contrast, the percentage of VLA-4 positively-stained CD8+ TILs was slightly lower than that of normal CD8+ T cells. Similarly, MFI for VLA-4 expression on CD8+ TILs was significantly lower than on normal CD8+ T cells. LFA-1 requires an active configuration to bind to its ligand, a process that can be induced by a variety of stimuli and NKI-L16 mAb reacts with a Ca2+-dependent activation epitope located on the ectodomain of α chain of LFA-1 (25–28). MFI and percentage of positively-stained cells for activated form of LFA-1 as recognized by NKI-L16 mAb were significantly higher on CD4+ TILs than on normal resting CD4+ T cells. NKI-L16 mAb staining was also slightly higher on CD8+ TILs than on normal CD8+ T cells. The majority of CD4+ and CD8+ TILs also highly expressed CD69 as well as CD25, both regarded as early activation markers. These results suggest that the spontaneous adhesion of TILs to osteoblasts and BMSCs depends on activated LFA-1 rather than on the amount of LFA-1 or VLA-4 expressed on TILs.

Spontaneous Polymerization of F-actin in TILs. Actin polymerization is a dynamic process that is critical for cellular adhesion. Functional LFA-1 is associated with polymerization of F-actin associated with intracellular domains of integrins (8, 29). Resting T cells seeded on fibronectin did not spread, and their F-actin content and distribution remained the same as observed by confocal microscopy (Fig. 6A). In contrast, a high expression of F-actin in the cell cortex and marked spreading and polymerization of F-actin were observed in PMA-activated T cells (Fig. 6B) and fresh unstimulated TILs (Fig. 6, C and D). These results suggest that polymerization of F-actin in TILs was spontaneously induced although the exact stimulus is not known at present.

Spontaneous Secretion of Chemokines MIP-1α and MIP-1β by TILs. We, as well as other investigators (6, 8, 30) have proposed that chemokines such as MIP-1α and MIP-1β functionally trigger T lymphocyte integrins. As shown in Fig. 7, unstimulated TILs produced significant amounts of MIP-1α and MIP-1β proteins in culture supernatants as well as in the cytosol, whereas PBTs did not produce any of these chemokines. These results suggest that MIP-1α and MIP-1β are spontaneously produced by TILs and may trigger integrin-mediated adhesion of TILs to osteoblasts and BMSCs in an autocrine manner.
Cellular Adhesion between TILs and Osteoblasts or Stromal Cells Induces IL-6 Production. Osteoblasts produce multiple soluble cytokines including IL-6 and IL-1β, which play an important role in bone metabolism (19, 31, 32). We also examined whether cellular adhesion between TILs and osteoblasts or BMSCs activates the latter cells to secrete IL-6. After 24 h culture, osteoblasts and BMSCs alone scarcely produced IL-6 (Fig. 8, A and B). However, the addition of formaldehyde-pretreated PBTs or TILs (with inhibited production of cytokine) to osteoblasts or BMSCs for 24 h resulted in a marked secretion of IL-6 from osteoblasts or BMSCs. More importantly, the concentration of IL-6 in the culture supernatants of TILs was significantly higher than in those containing PBTs. A partial inhibition of IL-6 secretion was produced by interference with cellular adhesion between TILs or PBTs and osteoblasts or BMSCs, using mAb against LFA-1 or VLA-4 but not control mAbs against MHC class-I and -II antigens. These findings suggest that TILs induce osteoclastogenic IL-6 secretion from osteoblasts or BMSCs through cellular adhesion via LFA-1/ICAM-1 and VLA-4/VCAM-1 pathways.

DISCUSSION

The major findings of the present study were that osteoblasts and BMSCs express at least two adhesion molecules, ICAM-1 and VCAM-1, and that osteoblasts and BMSCs specifically adhere to any opposing and heterotypic cells that possess specific receptors for these adhesion molecules. It is well known that cellular adhesion may potentially transduce activation of attached cells. Our results showed that the production of IL-6 from osteoblasts and BMSCs was amplified after cellular adhesion to TILs and that such activation was inhibited by mAbs against LFA-1 and VLA-4 on TILs. IL-6 is produced by osteoblasts and stimulates osteoclast function, which leads to bone resorption (19, 31, 32). Bone loss by osteoclast activation that is associated with inflammation has been reported previously (20). In addition, osteoclast activation and subsequent hypercalcemia are often observed in metastasis of tumors to the bone (33, 34). On the other hand, the production of many other cytokines is rather reduced by TILs (35), suggesting that cytokines that are produced by TILs may...
not be relevant to bone metabolism. It is possible that tumor cells may adhere to osteoblasts and BMSCs. However, the majority of tumor cells derived from solid tumors in the lung, breast, prostate, and colon do not express suitable adhesion receptors, LFA-1 and VLA-4, to ICAM-1 and VCAM-1 on osteoblasts or BMSCs, respectively. These features indicate that such tumor cells cannot adhere to osteoblasts and BMSCs. Therefore, cellular adhesion of osteoblasts and BMSCs to TILs may affect orderly events that lead to excessive production of osteoclastogenic cytokines locally, which enhance bone resorption, bone recruitment, and bone loss. TILs may accumulate within tumors and contribute to the development of cell-mediated responses as a result of tissue injury and repair (12, 13). In fact, certain chemotactic factors such as Mig (monokine induced by IFN-γ) and IP-10 (γ-IFN-induced peptide), secreted by cells within the tumor, are known to attract T cells to the neoplasm (36, 37). Other studies have also shown a strong adhesive properties for TILs toward the endothelium, a process that ultimately results in the accumulation of TILs in appropriate tumor milieu (38). On the basis of the present findings and the
results of previous studies, we propose the following two novel features of chemotaxis and adhesion of TILs in the bone and bone marrow that contain metastatic tumors: (a) integrins on TILs are spontaneously activated by endogenous chemokines MIP-1α and MIP-1β, which contribute to the highly adhesive characteristics of TILs; and (b) in bone or bone marrow milieu, TILs bring about an imbalance of bone metabolism by inducing excessive production of the osteoclastogenic cytokine, IL-6, through adhesion to osteoblasts or BMSCs.

T-cell integrins adhere firmly to their ligands that belong to the immunoglobulin superfamily, including ICAM-1 and VCAM-1, both of which were expressed on osteoblasts and BMSCs. However, integrins on resting PB Ts do not mediate such adhesion until activated by certain stimuli (4, 5, 39). In contrast, the present results showed that TILs obtained from patients with lung or breast cancer spontaneously adhered to osteoblasts and BMSCs without any exogenous stimulation. Furthermore, TILs expressed high levels of certain activation markers, namely CD25 and CD69, and the adhesion of TILs was further augmented by stimulation with PMA and a mixture of chemokines MIP-1α and MIP-1β, which suggests that TILs are in a state that is highly responsive to activation stimuli. In addition, TILs spontaneously expressed the activated form of integrin LFA-1, as recognized by NKI-L16 mAb, which reacts with a Ca²⁺-dependent activation epitope located on the ectodomain of the α chain of LFA-1 (25, 26). Active configuration of LFA-1 is thought to be induced by a conformational change of LFA-1 and/or clustering of the LFA-1 molecules (27–29, 40). Several studies have suggested that F-actin polymerization and integrins are involved in the activation of integrins (8, 41, 42). Our results showed a clear increase of F-actin in the cell cortex and a marked spreading, polymerization, and molecular rearrangement in unstimulated TILs. In contrast, the distribution of F-actin on
ADHESION OF TILS TO STROMAL CELLS

PBTs was stable in our study. Studies from our laboratories as well as those of others have previously demonstrated that chemokines MIP-1/β and IL-8 induce integrin-mediated adhesion of T cells and neutrophils, respectively (6, 43). TILs produced high quantities of both MIP-1α and MIP-1β in the culture supernatant as well as in the cytoplasmic fraction of TILs. In comparison, TILs produced only a small amount of tumor necrosis factor α and IL-1β (data not shown). Furthermore, the addition of exogenous mixture of MIP-1α and MIP-1β increased NK1-L16 mAb-binding on normal PBMCs, but it did not augment mAb-binding on TILs (data not shown). On the basis of these results, we suggest that endogenous MIP-1α and MIP-1β activate integrins on TILs in an autocrine manner and that cytoskeletal rearrangement may be involved in the activation of integrins and subsequent induction of adhesion of TILs to osteoblasts and BMSCs.

In conclusion, we showed that activated TILs spontaneously adhered to osteoblasts and BMSCs that bear appropriate integrin-ligands ICAM-1/VCAM-1. Such adhesion resulted in the production of IL-6 by osteoblasts or BMSCs. Our results also showed that most TILs were present within the supporting stroma of bone metastatic tumors rather than in the tumor parenchyma (Fig. 1) as demonstrated previously by other investigators (12, 44). Our results suggest that TILs may accumulate around tumors through cell-mediated responses. More importantly, within the bone or bone marrow milieu, TILs may potentially cause an imbalance in bone metabolism by favoring bone resorption through increased production of cytokines after adhesion to osteoblasts or stromal cells.

Fig. 7. Spontaneous production of MIP-1α and MIP-1β by TILs. The concentration of cytokines in culture supernatants (A, C) of normal PBTs (○) or TILs (●) after 24-h incubation at 37°C without any stimulation or in cytosol (B, D) of normal PBTs and TILs were determined by MIP-1α (A, B) and MIP-1β (C, D) ELISA system. Each point shows the concentration of MIP-1α and MIP-1β in the lysate or supernatant derived from 1 × 10⁵ cells of individual subjects. The mean ± SD data of each group are shown, together with the results of statistical analysis (Student’s t test).

Fig. 8. Adhesion of osteoblasts and BMSCs with normal PBTs or TILs induces IL-6 secretion. PMA-activated PBTs or unstimulated TILs were pre-treated with 3% formaldehyde for 2 h at 4°C. Culture supernatant was collected from osteoblasts (A) and BMSCs (B) that were incubated with or without PMA-activated PBTs or unstimulated TILs in the presence or absence of the indicated mAbs for 24 h at 37°C. The concentration of IL-6 was determined by IL-6 ELISA system.

4144
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REFERENCES

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