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Immunocompetent Properties of Human Osteoblasts: Interactions With T Lymphocytes

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ABSTRACT: We sought to determine whether osteoblasts (OBs) can serve as accessory cells (ACs) for T-cell activation and whether T cells directly activate OB production of IL-6, using primary human OBs (NHOst), the transformed fetal osteoblast line hFOB1.19, and an osteosarcoma line SaOS-2. Robust, bidirectional activating interactions were shown using each of these three human ostoblast lines.

Introduction: Osteoblasts (OBs) could come into contact with lymphocytes during inflammatory joint destruction and fracture repair.
Materials and Methods: We used several in vitro assays to assess the ability of T cells and OBs to interact in the generation of immune and inflammatory responses.
Results: By flow cytometry, three OB cell lines all were found to express ligands for T-cell co-stimulation. The integrin ligand CD54/ICAM-1 was constitutively expressed by hFOB1.19 and NHOst and was upregulated on SaOS-2 by IFN-γ. MHC Class II was upregulated on all three lines by IFN-γ. CD166/ALCAM, a ligand of the T-cell molecule CD6, was constitutively expressed on all three lines. A second putative CD6 ligand designated 3A11 was expressed on hFOB1.19 and NHOst, but not consistently on SaOS-2. The ectoenzyme CD26 (dipeptidyl peptidase IV) was expressed on hFOB1.19 and NHOst, but not on SaOS-2. All three cell lines presented superantigen to T cells, especially after treatment with IFN-γ. Superantigen presentation was inhibited by antibodies to the leukocyte integrin CD11a/CD18 (LFA-1), MHC Class II, and CD54/ICAM-1. T cells, particularly when cytokine activated for 7 days before co-culture, stimulated all three osteoblast lines to produce interleukin (IL)-6, and this effect was boosted when IL-17 was added to the co-cultures with either resting T cells or cytokine-activated T cells.
Conclusions: Bidirectional activating interactions are readily shown between human T cells and several types of human OBs. The expression by OBs of ligands for the T cell–specific molecule CD6, as well as other molecules involved in immune interactions, strongly suggests that such in vitro interactions are representative of physiologic or pathologic events that occur in vivo.
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Key words: inflammation, osteoblasts, cytokines, integrins, lymphocytes

INTRODUCTION

The osteoblast is a mesenchymal cell with a primary role in bone formation and remodeling.[1] The osteoblast and its secreted products, such as interleukin (IL)-6, maintain a complex and carefully balanced relationship with the osteoclast, a cell of hematopoietic origin that controls bone resorption. In addition, osteoblasts may come into contact with cells of the immune system under several circumstances, including bone erosion by invading synovial pannus in rheumatoid arthritis, lymphocytic inflammation of bone marrow adjacent to joints affected by inflammatory arthritis,[2] bone remodeling during fracture repair, invasion of bone by neoplasms that contain tumor-infiltrating lymphocytes,[3] and lymphoid development in regions of bone marrow directly adjacent to bone.

Previous studies have suggested that osteoblasts might be able to interact with both B and T lymphocytes, in a manner that could facilitate lymphocyte activation.[4,5] Moreover, expression of cell surface structures that are characteristic of cells of the immune system, including ligands for signaling molecules on the T-cell surface, has been documented on human osteoblast lines and in intact bone tissues.[4–12] Osteoblasts can also synthesize a variety of cytokines and chemokines,[13–16] and they express cytokine receptors that mediate responses to secreted products of activated lymphocytes.[16–19] However, it is not yet known whether osteoblasts can serve as antigen-presenting cells (APCs) that induce robust responses to specific antigens by resting T cells and whether primary human T cells can alter cytokine production by osteoblasts.

T lymphocytes can respond to processed peptide antigens, when presented by major histocompatibility complex

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molecules. This response is major histocompatibility complex (MHC)-restricted, requiring identity between MHC molecules on the APCs and self-MHC molecules to which T-lineage cells were exposed during their development in the thymus. This response requires co-stimulatory signals delivered by ligands on the surface of the APCs and/or secreted cytokines produced by the APCs and results in activation of a restricted set of T-cell clones. T cells can also respond to microbial superantigens, which bind to MHC molecules without prior processing into small peptides. Superantigens activate a broader range of T-cell clones, and, although presented by MHC, do not require MHC identity between the APCs and the responding T cells. Previous work from several laboratories, including our own, has shown that cells not conventionally regarded as APCs, such as synovial fibroblasts, can present superantigens to resting human T cells and support a vigorous T-cell proliferative response, in a manner that involves engagement of co-stimulatory ligands on the fibroblast by their receptors on the T-cell surface.[20] Moreover, resting T cells can activate synovial fibroblasts to produce pro-inflammatory cytokines and other mediators, even when antigens or mitogens that would activate the T cells are omitted from the co-cultures.[21]

These studies were performed to test several hypotheses: that a variety of human osteoblast populations express molecules characteristic of cells of the immune system, which are involved in the activation of lymphocytes by APCs; that expression of some of these molecules is regulated by IFN-γ; that osteoblasts, like synovial fibroblasts, can support T-cell activation by superantigens; and that T cells can augment production of IL-6 by human osteoblasts.

MATERIALS AND METHODS

Reagents and antibodies

Antibodies against CD2 (1E7E8), CD3 (UCHT1), CD4 (QS4120), CD6 (3F7B5), CD8 (UCHT4), CD14 (UCHM1), CD16 (3G8), CD19 (BU12), CD26 (202.36), CD58 (TS2/9), CD80 (P1.H5.A1.A1), CD86 (BU63), CD166 (3A6), and MHC Class II (TDR31.1) were purchased from Ancell Corp. Antibodies against CD11a (G43–25B), CD25 (M-A251), and CD54 (HA58) were purchased from Pharmingen Research Products. The 3A11 antibody, which recognizes a novel CD6 ligand and was recently described,[22] was made in the University of Michigan Hybridoma Core (Ann Arbor, MI, USA). Mouse IgG1 was purchased from Sigma Chemical (St Louis, MO, USA). The NS-1 nonsecreting hybridoma was purchased from American Type Culture Collection (Rockville, MD, USA). The secondary phycoerythrin (PE)-conjugated antibody goat anti-mouse IgG was purchased from Molecular Probes. IFN-γ was purchased from Pierce. The superantigen *Staphylococcus* enterotoxin A (SEA) and the lectin phytohemagglutinin (PHA) were purchased from Sigma Chemical. SEA and PHA stimulate T-cell proliferation in a manner dependent on the presence of accessory cells (also termed APCs). DMEM, Ham's F-12, McCoy's 5A, and Geneticin (G418) were purchased from Invitrogen. Osteoblast growth medium (OGM) medium was purchased from Cambrex. FBS was purchased from Atlanta Biologicals (Norcross, GA, USA). The cytokines IL-2 and IL-6 were purchased from Pharmingen, and TNF-α was purchased from Research Diagnostics. The human IL-6 OptEIA ELISA set was purchased from BD Biosciences.

Cell culture and T-cell preparation

T cells were prepared by adding an antibody cocktail comprised of CD14, CD16, CD19, and MHC Class II (Ancell) to peripheral blood mononuclear cells and negatively selecting the T cells using Biomag anti-mouse IgG magnetic beads (Qiagen). These T cells were routinely ≥99% pure as judged by flow cytometry and lacked sufficient accessory cells to support T-cell proliferation, unless such cells were added back to in vitro cultures. Cytokine-activated T cells were produced by culturing fresh T cells with IL-2, IL-6, and TNF-α for 7–9 days before use, as previously described.[23]

Osteosarcoma cell line SaOS-2 and the SV40 transfected osteoblast line hFOB1.19 were purchased from American Type Culture Collection, and the primary osteoblast line NHOst was purchased from Cambrex. The SaOS-2 line was grown in McCoy's 5A medium supplemented with 15% FBS, and the transformed hFOB1.19 line was grown in Ham's F-12 and DMEM with G418 and supplemented with 10% FBS. The primary osteoblast line NHOst was grown in a proprietary medium OGM purchased from Cambrex.

Flow cytometric analysis of osteoblast surface proteins

All three cell lines were analyzed for the presence of specific surface proteins using fluorescence-activated cell sorting (FACS) analysis. Briefly, aliquots of cells were incubated with antibodies against the surface proteins of interest for 30 minutes at 4°C and then washed twice in PBS containing 2% newborn calf serum. Some of these antibodies were directly conjugated with the fluorescent dye PE. If the antibody was not directly conjugated, a secondary antibody, PE-conjugated goat anti-mouse Ig, was added to those samples, incubated for an additional 30 minutes at 4°C, and washed twice in PBS containing 2% newborn calf serum. Negative controls, MsIg, or a nonspecific hybridoma supernatant, NS-1, were used to determine background fluorescence. Anti-CD98, a cell surface protein expressed on virtually all cultured cell lines, was used as a positive control to check for proper staining technique. All samples were fixed with 1% formalin. Samples were analyzed for fluorescence intensity using a Beckman Excel flow cytometer.

Superantigen presentation by osteoblasts and blocking assay

Each cell line was cultured until confluent. After irradiation (3000 rads in a Cesium irradiator), the cell lines were plated in 96-well plates with 10,000 (SaOS-2 and NHOst) or 20,000 cells/well (hFOB1.19) The cells were allowed to adhere for 48 h, after which 1000 units/ml of IFN-γ was added to some of the wells for another 48 h. Fresh medium was

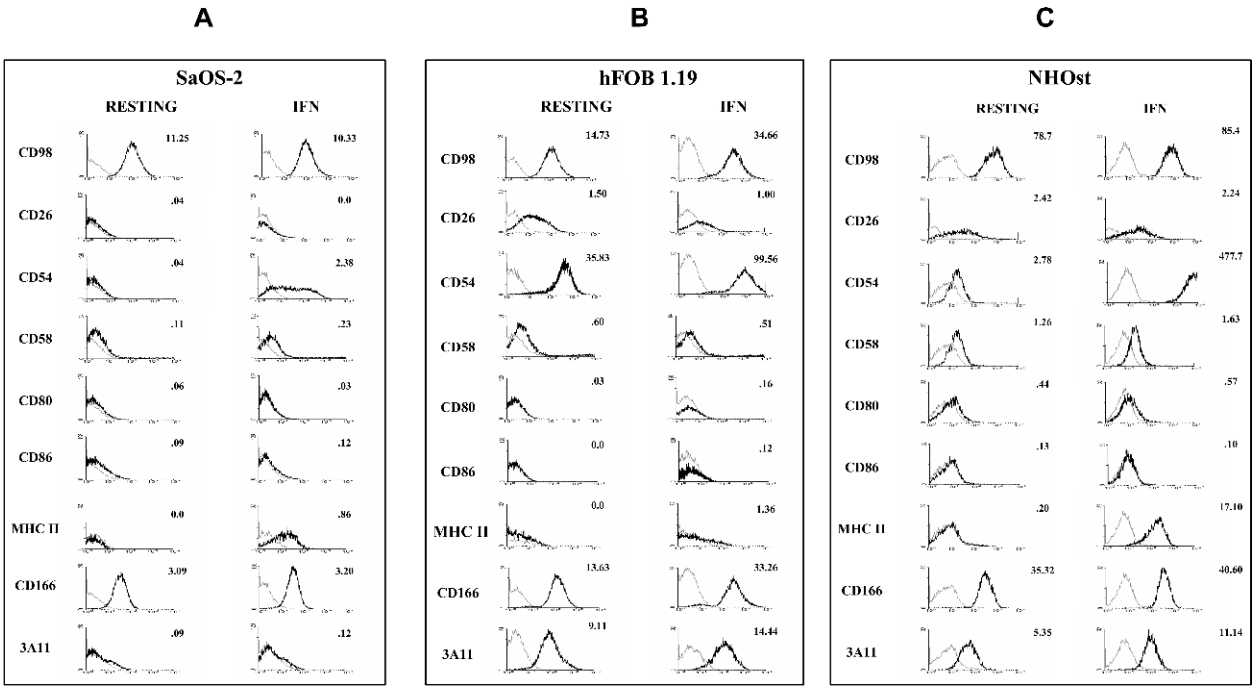


FIG. 1. Flow cytometric analysis of osteoblast cell lines for T cell–interacting molecules. (A) SaOS-2, (B) hFOB 1.19 cells, and (C) NHOst were treated with either media or IFN-γ (1000 U/ml) for 48 h. Direct staining was done with PE-conjugated antibodies to MHC Class II, CD54, and CD166, using MsIg as a negative control. Indirect staining was done with 3A11 mAb and FITC-goat anti-mouse Ab, using NS-1 as a negative control. Fluorescence intensity is measured on the *x*-axis on a logarithmic scale. The number on each histogram indicates the mean fluorescence intensity of the sample labeled with monoclonal antibody directed at the specific cell surface determinant. Shift of a fluorescence histogram to the right reflects higher cell surface expression of the molecule identified. The area under each fluorescence histogram corresponds to the number of cells analyzed for each sample.

added to the wells along with freshly prepared T cells (50,000 cells/well) with or without SEA and with or without candidate blocking reagents. The plates were cultured for 3 days after which [^{3}H]thymidine was added to each well for an additional 18 h. T-cell proliferation was assessed by measuring thymidine uptake using a Wallac Betaplate Reader (LKB Wallac).

Assay for IL-6

Osteoblast lines were set up in 6-well plates (3×10^5/well) and cultured either alone or with resting T cells (4×10^6/well), cytokine-activated T cells (4×10^6/well), IL-17, or a combination of T cells and IL-17. After 24 or 48 h, the supernatants were collected, and levels of IL-6 were assessed using a standard IL-6 ELISA kit from BD Biosciences.

RESULTS

Osteoblasts express immune system–associated cell interaction molecules

Given the expression of activated leukocyte cell adhesion molecule (ALCAM), a ligand for the T-cell molecule CD6, by stem cells in fetal perichondrium[24] and the ability of these cells to differentiate into osteoblasts, we wanted to study the possibility that osteoblasts might also express other molecules that would enable them to interact with the immune system. We used immunofluorescent staining and flow cytometry to detect the expression of a variety of such molecules by SaOS-2, hFOB 1.19, and NHOst cells under both resting and IFN-γ–treated conditions (Fig. 1). All three cell lines constitutively express the CD6 ligand CD166 (ALCAM), and hFOB 1.19 and NHOst also express 3A11, a putative CD6 ligand, in response to IFN-γ. hFOB 1.19 and NHOst constitutively express CD26, a serine peptidase and co-stimulatory molecule typically found on activated T cells. All three lines upregulate CD54 and Class II MHC in the presence of IFN-γ. Thus, osteoblasts have surface structures that could potentially allow them to interact with T cells.

Osteoblasts function as accessory cells for T-cell activation

Given the presence of the above molecules on the surface of osteoblasts, we next wanted to determine if these cells are capable of activating T cells. We measured the level of T-cell proliferation by [^{3}H]thymidine incorporation after co-culture with osteoblasts in the presence of superantigen or lectin. All three cell lines were able to present SEA to T cells, especially after treatment with IFN-γ (Figs. 2A–2C). T-cell proliferation in the presence of the lectin PHA was supported by either SaOS-2 or hFOB 1.19, regardless of IFN-γ treatment, to levels as high as 86,000 and 70,000 CPM, respectively (Fig. 3). Antibody blocking studies were performed to elucidate which molecular interactions are important for superantigen presentation. Monoclonal anti-

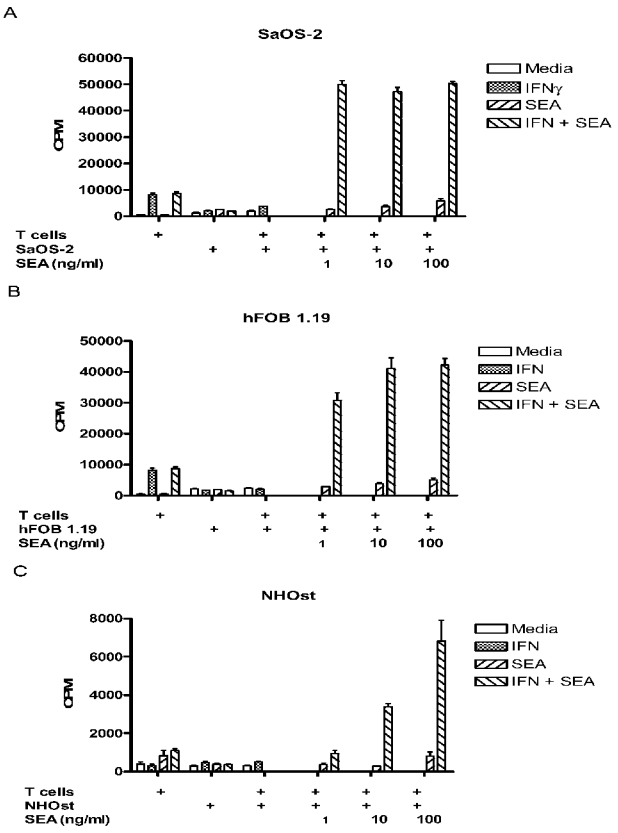


FIG. 2. Presentation of superantigen to T cells by osteoblasts. (A) SaOS-2, (B) hFOB 1.19, and (C) NHOst were treated with medium or IFN-γ (1000 U/ml) for 48 h. hFOB 1.19 and SaOS-2 but not NHOst were irradiated before T cells and SEA (1–100 ng/ml) were added and incubated for 72 h. Proliferation was measured by pulsing for 18 h with 3H-thymidine.

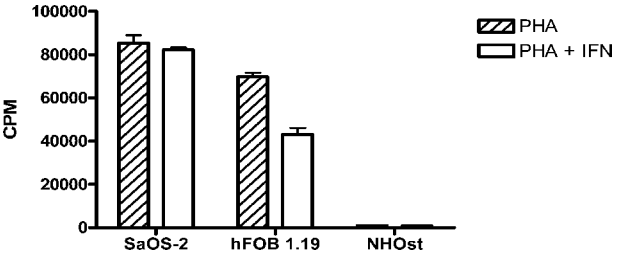


FIG. 3. PHA stimulation of superantigen to T cells with osteoblasts. SaOS-2, hFOB 1.19, and NHOst were treated with either medium or IFN-γ (1000 U/ml) for 48 h. hFOB 1.19 and SaOS-2 but not NHOst were irradiated before T cells and PHA (10 μg/ml) were added. The co-cultures were incubated for 72 h before pulsing with 3H-thymidine to measure proliferation.

bodies to MHC Class II, CD54, and CD11a significantly blocked stimulation of T-cell activation by all three cell lines in comparison with MsIg (Figs. 4A–4C). Anti-CD2 also somewhat inhibited the proliferation of T cells in response to osteoblast presentation of SEA by the three lines (Fig. 4). These data indicate that osteoblasts may function in antigen presentation, a process that is inhibited by the blockade of molecules known to play a role in interactions with T cells.

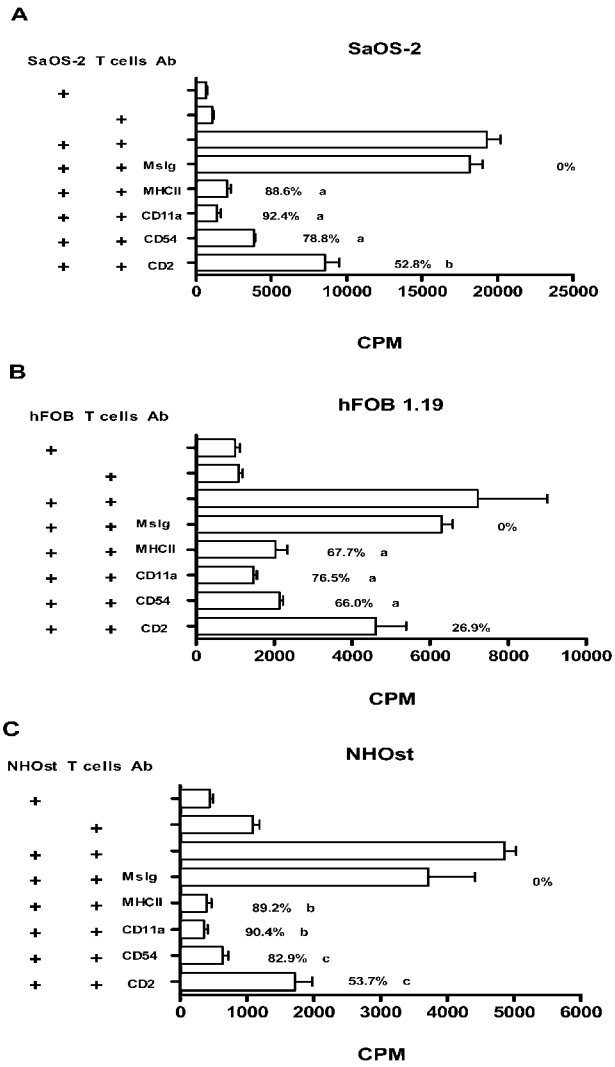


FIG. 4. Antibodies block superantigen presentation in osteoblasts. (A) SaOS-2, (B) hFOB 1.19, and (C) NHOst were treated with SEA (10 ng/ml) and T cells that had been incubated with blocking antibodies (20 ng/ml) for 1 h. The co-cultures were incubated for 72 h before pulsing with 3H-thymidine to measure proliferation. Percent inhibition is shown compared with osteoblasts with T cells + MsIg. Significant differences were determined by Student's *t*-test and are indicated by $^{a}p < 0.001$, $^{b}p < 0.01$, and $^{c}p < 0.05$.

Osteoblasts augment IL-6 production when stimulated by T cells or IL-17

We next asked whether the interaction between osteoblasts and T cells could result in mutual activation, not only causing T-cell proliferation but also eliciting secretion of bone resorptive cytokines from osteoblasts. We therefore measured osteoblast IL-6 production by ELISA after culture with T cells and/or IL-17, a T-cell cytokine produced in vivo by rheumatoid arthritis synovium. hFOB 1.19 cells produced IL-6 in response to IL-17 treatment. Co-culture with T cells alone resulted in IL-6 concentrations only slightly above background, but when the T cells were preactivated with a cytokine cocktail or when IL-17 was added

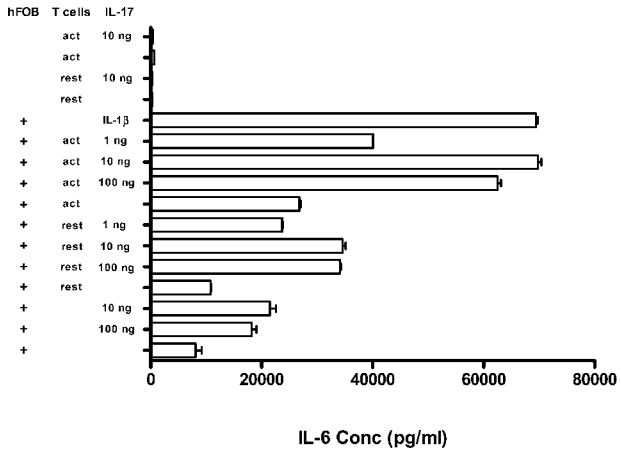


FIG. 5. Induction of osteoblast IL-6 production. The osteoblast cell line hFOB 1.19 and either resting T cells or cytokine-activated T cells were treated with varying concentrations of IL-17 (1–100 ng/ml) or IL-1β (1 ng/ml). IL-6 production was measured by ELISA. Similar results were obtained using primary osteoblast NHOst cells or osteosarcoma SaOS-2 cells.

to the co-culture, significant IL-6 secretion occurred. The highest levels of IL-6 were secreted in the presence of both cytokine-activated T cells and IL-17. This response to IL-17 was dose-dependent (Fig. 5). Similar results were seen for SaOS-2 and NHOst (data not shown).

DISCUSSION

There is a growing body of literature that suggests that osteoblasts are capable of active participation in interactions with the immune system. Such interactions would likely play an important role in physiologic functions, including the response to bone infection[12] and traumatic injury, as well as support of hematopoietic progenitor cells.[9] Furthermore, in pathological conditions such as rheumatoid arthritis and bony metastases, osteoblasts could contribute to the maintenance of the immune response. Given the bone loss common to both of these situations, the interplay between osteoblasts and immune cells likely stimulates increased bone resorption. Thus, our studies were intended to assess the expression of immunoreactive molecules on the surface of osteoblasts, the ability of the osteoblasts to stimulate T cells, and the induction of cytokine secretion from osteoblasts by T cells.

Previous studies have detected a variety of molecules on osteoblasts typically associated with APCs. CD54, also known as ICAM-1, is a cell surface glycoprotein of the immunoglobulin family expressed constitutively on endothelium and some lymphocytes and monocytes and on many cell types after stimulation with IL-1, TNF-α, IFN-γ, and lipopolysaccharide (LPS). This molecule binds to CD11a/CD18 (LFA-1) and is important for leukocyte–leukocyte, leukocyte–endothelium, and leukocyte–epithelium interactions. The interaction of CD54 on APCs and CD11a/CD18 on T cells is essential for T-cell activation.[25] Our study found CD54 on SaOS-2, hFOB 1.19, and NHOst, in agreement with earlier studies of both mouse and human osteoblastic cell lines.[3,4,8,10,26] Previous work did not detect CD54 on the osteosarcoma line U2-OS,[9] a surprising result given its presence on the osteosarcoma line SaOS-2, but this finding is likely caused by the lack of IFN-γ stimulation used in that experiment, which we have shown to be important for the upregulation of this molecule. CD58, or LFA-3, is a structure that interacts with CD2 on T cells, thus mediating binding of T cells to APCs and endothelium.[27] No previous studies have found CD58 on the surface of osteoblasts, and we have not shown any significant expression of it on any of the three cell lines we studied.

For T cells to become activated, they must not only recognize the antigen, but also receive a co-stimulatory signal or signals from an APC. Several molecules, including CD80 and CD86, are known to be important for this process. These molecules are found on APCs and bind to CD28 or CD152 on T cells to produce stimulatory or inhibitory signals, respectively.[28] Neither of these molecules is significantly expressed on SaOS-2, hFOB 1.19, or NHOst, in contrast to previous work showing both CD80 and CD86 on cultured human osteoblasts.[10]

ALCAM and 3A11 are also molecules with known T-cell interactions. CD6 is a T-cell molecule with a role in adhesion to thymic epithelium and likely in T-cell activation.[29] ALCAM (CD166) is a known ligand of CD6 found on PHA-activated peripheral blood mononuclear cells (PBMCs) as well as a variety of other cell types.[30] Blocking the CD166 binding domain of CD6 has been found to decrease antigen-dependent T-cell proliferation.[31] CD166 is constitutively expressed on all three cell lines we studied as it is on the U2-OS line.[9] Our results also include the novel finding that IFN-γ upregulated 3A11, a novel second CD6 ligand,[22] on hFOB 1.19 and NHOst cells. This second CD6 ligand is generally absent from bone marrow–derived cells but is expressed by synovial fibroblasts and keratinocytes.[22]

Rather than responding to soluble antigens, T cells only become activated when antigens are presented by self-MHC on the surface of the APC. MHC Class I is found on all nucleated cells and interacts with CD8 + T cells, whereas MHC Class II is typically found on APCs and interacts with CD4 + T cells. Our findings confirm the IFN-γ stimulated expression of MHC Class II on human osteoblasts.[7,10]

CD26 is a 110-kDa cell surface glycoprotein with serine protease activity found on activated T cells, macrophage subsets, and specialized fibroblasts of the skin, mammary glands, and synovia.[32,33] It is capable of cleaving cytokines and chemokines and also acts as a co-mitogenetic signal in the CD3/TCR and CD2 T-cell activation pathways.[33] We have found that hFOB 1.19 and NHOst cells constitutively express CD26, a molecule not previously known to be present on osteoblasts.

The presence of these immunomodulatory structures implies that osteoblasts are potentially capable of antigen presentation. In addition to this evidence, previous studies have also suggested that osteoblasts may stimulate T-cell proliferation. For instance, culture medium conditioned by rat osteosarcoma (ROS) cells has been shown to induce the proliferation of HT-2 murine T cells, especially after the

ROS cells were treated with PTH or LPS.[15] In addition, human osteoblasts increase the proliferation of IL-2–activated T cells, an effect that is enhanced by treatment of the osteoblasts with IFN-γ and TNF-α.[4] Another study used human osteoblasts to stimulate allogeneic PBMCs as well as activate nonadherent autologous PBMCs in the presence of purified protein derivative (PPD).[5] Although the nonadherent fraction of PBMCs most likely contains largely T cells, the potential for contamination by known APCs without more rigorous purification of the T cells prevents this study from being a conclusive demonstration of antigen presentation by osteoblasts. Our study is the first to show that osteoblasts can support a proliferative response of T cells to presentation of a superantigen (by SaOS-2, hFOB 1.19, and NHOst osteoblastic lines) and lectin (by SaOS-2 and hFOB 1.19 cells). Even at concentrations as low as 1 ng/ml of the superantigen SEA, a proliferative response was detected. This response is likely of physiological significance given the evidence that superantigens are important in autoimmune diseases such as rheumatoid arthritis,[34] and the role of *S. aureus*, a bacterium that elaborates several superantigens, in osteomyelitis.

Although superantigen acts by linking the T-cell receptor to MHC Class II on an APC, thus stimulating proliferation of the T cell, MHC Class II is not sufficient for this action. Previous studies have found cells expressing MHC Class II that are incapable of supporting this response.[35] Furthermore, we have shown that SEA-induced T-cell proliferation is inhibited by blocking not only MHC Class II, but also CD54, CD11a, and CD2. The effect of anti-CD2 likely represents the generation of an anti-proliferative signal by the anti-CD2 antibody rather than inhibition of ligand engagement because the known human CD2 ligand CD58 was absent from osteoblasts. Although activation of T cells by osteoblasts has similar requirements in some respects compared with traditional APCs, the absence of the CD28 ligands CD80 and CD86 from osteoblasts points out differences from classic APCs, and similarities to APC function of fibroblasts.[20] In addition, the decreased ability of the NHOst cells to support a lectin response in comparison with the SaOS-2 and hFOB 1.19 lines implies a differential expression of immune-associated molecules by these lines. This finding is further evidence that the antigen-presenting capabilities of osteoblasts cannot be explained simply by the presence of MHC Class II on their surface. The explanation for the inability of NHOst to support lectin responses is unclear at this time. PHA interacts with a variety of molecules on the lymphocyte surface, and likely also on the accessory cell as well, but the full scope of these binding interactions is not yet understood.

The interaction between T cells and osteoblasts could contribute to the bone resorption seen in diseases such as rheumatoid arthritis and osteomyelitis if such interactions cause cytokines to be produced that are supportive of tissue destruction. IL-6 is a cytokine produced by several cell types, including T cells, monocytes, and fibroblasts, with pro-inflammatory as well as osteoclast-inducing effects.[36] IL-6 is abundant in the synovial tissue of patients with rheumatoid arthritis,[37] and has previously been shown to be produced by osteoblasts in response to TNF-α or IL-1 stimulation, ICAM-mediated adhesion to tumor-infiltrating lymphocytes, co-culture with activated T cells, and infection by *Salmonella* or *S. aureus*.[3,36,38,39] Our data indicate that SaOS-2, hFOB 1.19, and NHOst cells are capable of producing IL-6 when in contact with activated T cells. IL-17, a pro-inflammatory T-cell cytokine found in high levels in rheumatoid arthritis synovial fluid,[40] has previously been found to enhance IL-6 production in MC3T3-E1 mouse osteoblastic cells in concert with TNF-α prostaglandin F $(PGF)_{2\alpha}$, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (phorbol myristate acetate [PMA]), or endothelin-1, but not to have effects on IL-6 synthesis in isolation.[41–43] We have shown, however, robust dose-dependent IL-6 production by the human osteoblastic lines SaOS-2, hFOB 1.19, and NHOst in response to IL-17 alone. Our data indicate that activated T cells and IL-17 have additive effects on the elaboration of IL-6 by osteoblasts. Thus, the juxtaposition of osteoblasts, activated T cells, and high concentrations of IL-17 seen in rheumatoid arthritis creates a prime situation for the bone loss characteristic of this disease. TNF-α and other unidentified cytokines produced by activated T cells may also contribute to the stimulation of IL-6 synthesis by osteoblasts.[38]

In conclusion, we found that osteoblasts possess several immunocompetent properties, including the expression of immunoreactive structures on their surface, the presentation of superantigen to T cells, and secretion of a bone resorption–promoting cytokine in response to stimulation by T cells and IL-17. These findings could have clinical implications for pathological situations in which osteoblasts and T cells come into contact, including rheumatoid arthritis and osteomyelitis. A greater understanding of the unique immune ligand profile of osteoblasts, particularly the presence of more restricted molecules such as 3A11, and the contribution of the individual ligands to antigen presentation by osteoblasts could prompt development of therapies targeted to such molecules to inhibit continued T-cell activation in such situations. Our data also imply that IL-17 blockade could help preserve bone when osteoblast–immune system interactions occur, given the strong induction of IL-6 secretion by IL-17. This pathway for induction of bone loss could be maintained even in the presence of TNF-α inhibition, a therapeutic strategy that is often, but not always, effective in the treatment of rheumatoid arthritis.

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