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IL-1β Inhibits Human Osteoblast Migration

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Bone has a high capacity for self-renewal and repair. Prolonged local secretion of interleukin 1β (IL-1β), however, is known to be associated with severe bone loss and delayed fracture healing. Since induction of bone resorption by IL-1β may not sufficiently explain these pathologic processes, we investigated, *in vitro,* if and how IL-1β affects migration of multipotent mesenchymal stromal cells (MSC) or osteoblasts. We found that homogenous exposure to IL-1β significantly diminished both nondirectional migration and site-directed migration toward the chemotactic factors platelet-derived growth factor (PDGF)-BB and insulinlike growth factor 1 (IGF-1) in osteoblasts. Exposure to a concentration gradient of IL-1β induced an even stronger inhibition of migration and completely abolished the migratory response of osteoblasts toward PDGF-BB, IGF-1, vascular endothelial growth factor A (VEGF-A) and the complement factor C5a. IL-1β induced extracellular signal-regulated kinases 1 and 2 (ERK1/2) and c-Jun N-terminal kinases (JNK) activation and inhibition of these signaling pathways suggested an involvement in the IL-1β effects on osteoblast migration. In contrast, basal migration of MSC and their migratory activity toward PDGF-BB was found to be unaffected by IL-1β. These results indicate that the presence of IL-1β leads to impaired recruitment of osteoblasts which might influence early stages of fracture healing and could have pathological relevance for bone remodeling in inflammatory bone disease.
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INTRODUCTION

Bone is a highly metabolic tissue with a high rate of self-renewal by bone remodeling and the potency for repair without forming scar tissue (1). Formation of bone during embryonic development or after fracture can occur via intramembranous or endochondral ossification. In case of fracture healing, bone formation processes are initiated in the fracture hematoma during the early inflammatory phase in which inflammatory cytokines and various growth factors such as growth factor β superfamily members and angiogenic factors are released (2,3). The concerted action of these factors regulates the recruitment and proliferation of multipotent mesenchymal stromal cells (MSC) and osteoblasts, the differentiation of MSC into osteoblasts and chondrocytes as well as angiogenesis to reestablish a sufficient blood supply (2,3). After the formation of primary bone, remodeling leads to the formation of secondary bone, which depicts the original anatomical and functional properties before the fracture (2–4).

Since recruitment of MSC and osteoblasts is indispensable for fracture healing and life-long bone remodeling, several studies have focused on growth factors that are present in the fracture hematoma or deposited in bone and released upon bone resorption through osteoclasts as being chemotactic agents for osteoblasts and MSC. The platelet-derived growth factor β (PDGF-BB) was found to be a very potent chemotactic factor for MSC, primary osteoblasts and osteoblastic cell lines (5–10). Further examples are the insulinlike growth factors 1 (IGF-1) and 2 (IGF-2), which stimulate the site-directed migration of MSC and osteoblasts (5,11,12). In addition to its known angiogenic effects, vascular endothelial growth factor A (VEGF-A) was found to have a dose-dependent chemotactic effect on MSC and primary human osteoblasts, *in vitro*, that is mediated through VEGF receptor 1 activation (13,14).

In recent years, it has become clear that, in addition to growth factors, cells of the immune system, as well as cytokines and the complement system, have a profound impact on bone formation, bone remodeling and skeletal disorders (15,16). Mainly, excessive bone loss through osteoclasts observed in inflammatory and autoimmune diseases as well as the discovery of T cells and cytokines inducing osteoclastogenesis led to a rapidly emerging field called osteoimmunology (17). The complement factor C5a, locally released at sites of tissue damage and inflammation, has been reported to have a chemotactic effect on MSC (18). In contrast, MSC were recently found to

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express rather low levels of C5a receptor (C5aR) mRNA and to migrate toward C5a significantly less than osteoblasts, which show a higher C5aR mRNA expression (19).

A prominent cytokine released in the bone under various circumstances is IL-1β. In the fracture hematoma, IL-1β is released by macrophages together with tumor necrosis factor-α (TNF-α) and IL-6 to promote the recruitment of other inflammatory cells and initiate the repair process (20). IL-1β as well as IL-6 and TNF-α expression levels in the fracture hematoma were found to peak within the first 24-h after fracture and to decline rapidly afterward (20).

While short periods of IL-1β secretion are critical for successful fracture healing (20), persistent release of IL-1β can be associated with severe bone loss. In idiopathic osteoporosis patients, IL-1β release by peripheral blood monocytes, precursors of osteoclasts, was shown to be increased significantly when compared with healthy individuals (21,22). The notion of IL-1-mediated bone loss also is supported by a study where inhibition of IL-1 with soluble IL-1R was shown to significantly reduce inflammation, loss of connective tissue and bone resorption in the periodontium of *Macaca fascicularis* primates infected with pathogens (23). A different study showed that *IL1*$^{-/-}$h*TNF*tg mice were completely protected from systemic bone loss mediated through the overexpression of TNF-α (24). A possible pathogenetic role for IL-1β in bone loss is further supported by the fact that IL-1β enables peripheral blood mononuclear cells to differentiate into osteoclasts in the presence of osteoblastic SaOS-2 cells without further stimulation (25). In murine osteoblasts, IL-1β induced receptor activator of NF-κB ligand (RANKL) secretion while reducing osteoprotegerin (OPG) production and activated osteoclastogenesis (26). A subsequent study even found IL-1β to be essential for osteoclast formation as IL-1 receptor antagonist (IL-1RA) treatment inhibited RANKL/TNF-α–induced osteoclastogenesis (27).

Taken together, IL-1β has been shown to influence fracture repair and bone remodeling. The best studied function of IL-1β in bone metabolism is the activation of osteoclasts. Little, however, is known about the effects of IL-1β on migration of human osteoblast and MSC that are important for bone remodeling and repair. Therefore, we investigated in a human *in vitro* model if IL-1β has a chemotactic effect on osteoblasts and MSC or influences the chemotactic effect of other soluble factors active for osteoblast and MSC recruitment.

MATERIALS AND METHODS

Primary Osteoblast and MSC Isolation and Culture

Human osteoblasts were harvested from cancellous bone obtained during routine surgical procedures (total knee replacements) with informed consent of the patients and according to the requirements of the Ethics Committee at the University of Ulm. As described earlier (13), 5 g cancellous bone were broken up in small pieces, washed with phosphate-buffered saline (PBS) and incubated in Dulbecco's modified Eagle medium (DMEM) with 0.05% collagenase (Sigma-Aldrich, Schnelldorf, Germany) for 2 h at 37°C. After removing the supernatant and washing twice with PBS, bone pieces were cultured in 6-well plates with Ham's F-12 medium supplemented with 10 % fetal calf serum (FCS), 100 U/100 μg/mL penicillin/streptomycin, 2.5 μg/mL amphotericin B and 2 mmol/L L-glutamine (all Biochrom, Berlin, Germany) at 37°C, 95% humidity and 5% CO_2. Medium was changed twice a week. After one week, osteoblasts started to grow out, which were then transferred into culture flasks and passaged at maximum 3 to 4 times in DMEM supplemented with 10% FCS, 100 U/100 μg/mL penicillin/streptomycin and 2 mmol/L L-glutamine (all Biochrom) before application in chemotaxis, adhesion and MTT assays. The cell culture technique preserves the osteoblastic phenotype as described previously (28).

Human MSC were harvested from bone marrow obtained during routine surgical procedures (triple osteotomy) with informed consent of the patients and according to the requirements of the Ethics Committee at the University of Ulm. As described earlier (6), mononuclear cells were harvested from the interphase after a density gradient centrifugation (600*g*) with Biocoll (Biochrom), washed with PBS and resuspended in DMEM supplemented with 10% FCS, 100 U/100 μg/mL penicillin/streptomycin and 2 mmol/L L-glutamine (all Biochrom). Mononuclear cells were given into 75-cm^2 cell culture flasks and incubated at 37°C, 95% humidity and 5% CO_2. Nonadherent cells were washed off 24 h later and 10 ng/mL fibroblast growth factor-2 was added to the medium. Medium was changed twice a week. MSC were split at approximately 60% confluency and used in passage 3-6.

Soluble Factors and Inhibitors Employed in Chemotaxis and Adhesion Assays

For chemotaxis and adhesion assays, the following factors were used. Recombinant human PDGF-BB, VEGF-A, IGF-1 and IL-1β were purchased from Peprotech, Hamburg, Germany. Recombinant human C5a was purchased from Sigma-Aldrich. For ERK1/2 and JNK inhibition, PD 98059 and JNK inhibitor II (both Callbiochem, Darmstadt, Germany) were used, respectively.

Chemotaxis and Adhesion Assay

In vitro adhesion and chemotaxis assays were performed in a modified Boyden chamber (NeuroProbe, Gaithersburg, MD, USA) using polycarbonate filters with 8-μm pores between the lower well containing the chemotactic factor and the upper well containing osteoblasts. To evaluate the influence of IL-1β on the adhesion and chemotaxis of osteoblasts, IL-1β was either added only into the lower chamber to establish an IL-1β gradient or to both upper and lower chamber to create a uniform IL-1β concentration. Growth factors and cytokines were di-

luted in serum-free DMEM at concentrations that were found effective in previous studies (6,11,13,19): 10, 100 or 1000 pg/mL IL-1β, 100 ng/mL C5a, 10ng/mL PDGF-BB, 100 ng/mL IGF-1 and 100 ng/mL VEGF-A and filled in quadruplicates into the lower wells of the Boyden chamber, which was then covered by the polycarbonate filter. For negative control, DMEM alone was added to the lower well. Osteoblasts of passage 3-4 were trypsinized, washed and resuspended in serum-free DMEM. For the adhesion assay, 5×10^2 osteoblasts in 50 µL serum-free DMEM were given into the upper well of the Boyden chamber and incubated for 30, 60 and 90 min. In case of uniform IL-1β concentrations in upper and lower well of the Boyden chamber, IL-1β also was added to the cell suspension at 100 pg/mL. Adherent cells on the upper side of the polycarbonate filter were fixed, stained with Giemsa (Merck, Darmstadt, Germany) and counted.

For chemotaxis assay, 10^4 osteoblasts in 50 µL serum-free DMEM were given into the upper wells of the Boyden chamber. In case of uniform IL-1β concentrations in upper and lower wells of the Boyden chamber, IL-1β was added to the cell suspension at the appropriate concentration. For inhibition of chemotaxis with mitogen-activated protein kinase (MAPK) inhibitors, 10 µmol/L PD 98059 (ERK1/2 inhibition) or JNK inhibitor II (JNK inhibition) were given in both upper and lower wells of the Boyden chamber. After 4 h of incubation, the filter was obtained and adherent, nonmigrated cells on the upper side were scraped off with PBS and a rubber scraper. The migrated cells on the lower side were stained with Giemsa staining after fixation in a 4% formaldehyde solution. All migrated cells were counted at 20× magnification.

Immunocytochemistry of MSC and Osteoblasts

To detect IL-1 receptor type I (IL-1R1) expression in cultured MSC and osteoblasts, cells were seeded at 10^4 cells per well on 4-well glass slides with polystyrene vessels (BD Falcon) in complete medium and left overnight to adhere. Serum-free medium was added 30 min before staining. For detection of IL-1R1 expression in migrating MSC and osteoblasts, chemotaxis toward PDGF-BB or without chemoattractant was performed as described above. After migration, nonmigrated cells on the upper side were scraped off with PBS and a rubber scraper. Both the culture slides and the chemotaxis filter were fixed in a 4% formaldehyde solution and cells were stained with 2 µg/mL anti-IL-1R1 antibody (AF269; R&D Systems, Wiesbaden, Germany) and the Dako LSAB + System-HRP (K0690; Dako, Hamburg, Germany) according to the manufacturer's protocol. Negative controls were stained without exposure to the primary antibody (anti-IL-1R1).

Human Phospho-MAPK Array of IL-1β-Stimulated Osteoblasts

To determine the phosphorylation profile of MAPK in IL-1β-stimulated osteoblasts, we performed a Human Phospho-MAPK Array from R&D Systems. Osteoblasts were seeded at 1.5×10^6 cells per 10-cm cell culture dish and incubated in complete medium overnight to adhere. After serum-free medium was added, the control was left untreated while 100 pg/mL IL-1β was added to the other dish. After 30 min of incubation, cells were lysed and the protein content of both the control and the IL-1β treated sample was measured with the NanoDrop ND 1000 (Peqlab, Erlangen, Germany) at 280 nm to be of equal quantity. The array procedure was performed according to the manufacturer's protocol and spots were detected with the LAS-4000 (GE Healthcare, Munich, Germany).

Statistics

All data are presented as the mean of independent donors. Error bars represent standard error of the mean (SEM). Chemotaxis results were analyzed for their significance with two-way analysis of variance (ANOVA) followed by a Bonferroni *post hoc* test using GraphPad Prism 5 (GraphPad Software).

RESULTS

Effect of IL-1β on Cell Migration of Osteoblasts and MSC

Osteoblasts have the ability to migrate, both nondirectional and into the direction of a chemotactic gradient. When different concentrations of IL-1β were added to both upper and lower well of the Boyden chamber, significantly less osteoblasts migrated through the filter independent of the IL-1β dose between 10 and 1000 pg/mL as shown in Figure 1A. This reduced migratory activity also was observed when IL-1β was filled only into the lower chamber (Figure 1A), indicating that IL-1β had no chemotactic effect on osteoblasts, but actively diminished their migratory function.

In Figure 1B, IL-1β also was shown to actively abolish the chemotactic effect of the complement factor C5a in a dose-independent manner when present only in the lower chamber, but not in the upper. For the growth factors PDGF-BB and IGF-1, which are strong chemoattractants for osteoblasts (5–12), IL-1β was able to significantly reduce chemotaxis when filled into both upper and lower well (Figure 2A). Furthermore, the chemotactic effect of PDGF-BB, IGF-1 and VEGF-A was reduced below basal levels when IL-1β was filled only into the lower well of the Boyden chamber (Figure 2B).

To investigate if the reduced migratory function of osteoblasts in the presence of IL-1β is due to impaired adhesion through IL-1β, osteoblast adhesion to the filter was measured. Adhesion was found to be equal in serum-free medium as well as with IL-1β in only the lower or both in upper and lower well of the Boyden chamber as seen in Figure 3 indicating that the reduced migration of osteoblasts through IL-1β was not due to impaired adhesion.

In contrast to osteoblasts, MSC migration was not inhibited through IL-1β as

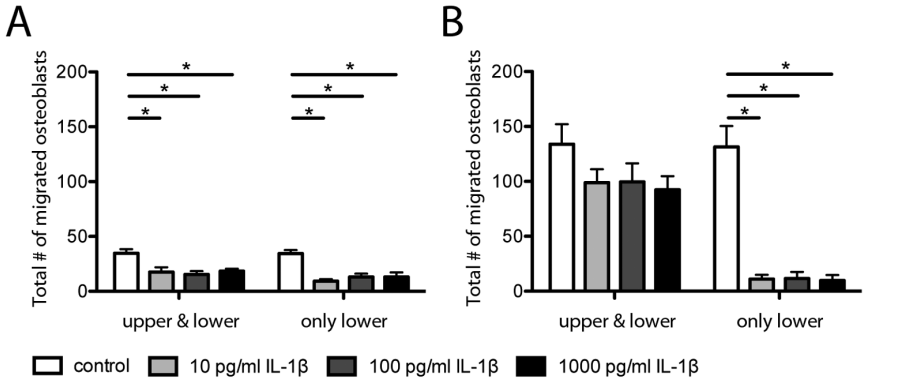

Figure 1. Influence of IL-1β on osteoblast migration under basal conditions (A) and toward C5a (B). *In vitro* migration was analyzed in a modified Boyden chamber using 8 μm polycarbonate filters. IL-1β was diluted in serum-free DMEM at 10 (light gray bars), 100 (dark gray bars) or 1000 (black bars) pg/mL and added either to both upper and lower or only to lower wells of the Boyden chamber as indicated. Serum-free DMEM was used as control (white bars). C5a was diluted in serum-free DMEM at 100 ng/mL. Columns represent mean number of totally migrated cells, bars represent SEM from three independent donors. Significance was calculated with two-way ANOVA followed by a Bonferroni *post hoc* test; $*P < 0.05$. (A) Migration of osteoblasts with IL-1β in upper and lower (left) or only in lower wells (right). (B) Migration of osteoblasts toward a C5a gradient with IL-1β in upper and lower (left) or only in lower wells (right).

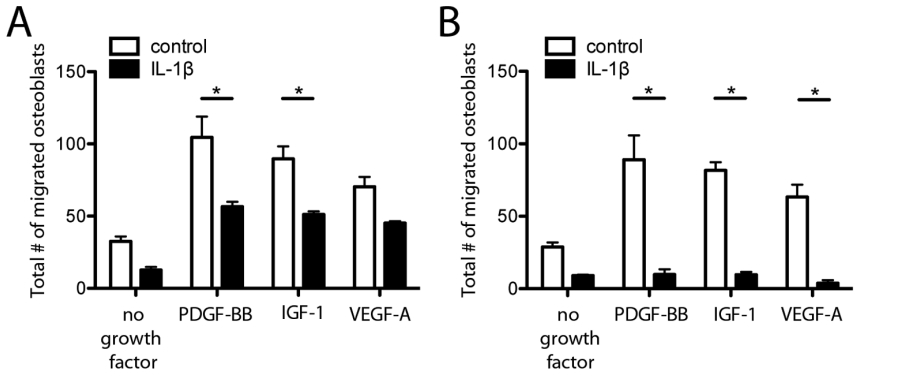

Figure 2. Influence of IL-1β on osteoblast migration toward several growth factors with IL-1β present in a uniform concentration (A) or as a gradient (B). *In vitro* migration was analyzed in a modified Boyden chamber using 8 μm polycarbonate filters. IL-1β was diluted in serum-free DMEM 100 pg/mL (black bars) and added either to both upper and lower (A) or only to lower (B) wells of the Boyden chamber as indicated. Serum-free DMEM was used as control (white bars). Growth factors were diluted in serum-free medium at the following concentrations: PDGF-BB (10 ng/mL), IGF-1 (100 ng/mL) and VEGF-A (100 ng/mL). Columns represent mean number of totally migrated cells, bars represent SEM from three independent donors. Significance was calculated with two-way ANOVA followed by a Bonferroni *post hoc* test; $*P < 0.05$. (A) Migration of osteoblasts toward growth factors with IL-1β in upper and lower wells. (B) Migration of osteoblasts toward growth factors with IL-1β only in lower wells.

seen in Figure 4. Furthermore, IL-1β did not influence migration of MSC in the presence of PDGF-BB indicating that IL-1β had no effect on MSC migration.

Expression of IL-1R1 in MSC and Osteoblasts

Since MSC migration, in contrast to osteoblast migration, was not inhibited through IL-1β, we investigated if MSC and osteoblasts both express the IL-1R1. We performed immunocytochemistry for IL-1R1 on adherent MSC and osteoblasts on cell culture glass slides and found a positive staining for both MSC and osteoblasts (Figures 5A, B, respectively) while negative controls did not stain positive (Figures 5C, D, respectively). Furthermore we could show that MSC and osteoblasts which had migrated toward PDGF-BB (Figures 5E, F, respectively) or without chemoattractant (Figures 5G, H, respectively) also express IL-1R1.

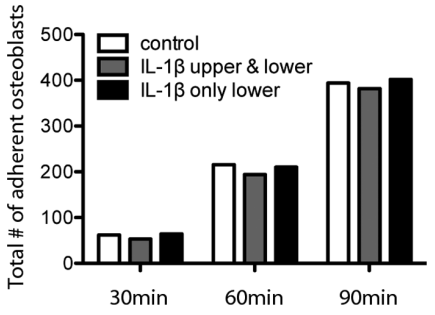

Figure 3. Influence of IL-1β on osteoblast adhesion. *In vitro* adhesion assay was performed in a modified Boyden chamber using 8 μm polycarbonate filters. IL-1β was diluted in serum-free DMEM at 100 pg/mL and added either to both upper and lower (gray bars) or only to lower wells (black bars) of the Boyden chamber as indicated. Serum-free DMEM was used as negative control (white bars). Columns represent mean number of adhered cells from one donor.

Phosphorylation Profile of MAPK in IL-1β Treated Osteoblasts

To identify signaling pathways involved in the IL-1β-induced inhibition of migration in osteoblasts, we investigated the phosphorylation profile of MAPK in osteoblasts stimulated with IL-1β. As seen in Figure 6, we found ERK1 and 2 as well as all JNK to be highly phosphorylated in osteoblasts after stimulation with IL-1β, which supports the finding that the IL-1β-induced inhibition of migration is dependent on ERK1/2 and JNK signaling. In addition, we also found an IL-1β-induced phosphorylation of p38α and p38γ MAPK.

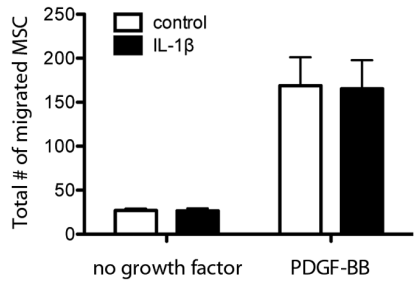

Figure 4. Influence of an IL-1β gradient on MSC migration toward PDGF-BB. *In vitro* migration was analyzed in a modified Boyden chamber using 8 μm polycarbonate filters. IL-1β was diluted in serum-free DMEM at 100 pg/mL and added to the lower wells of the Boyden chamber as indicated (black bars). Serum-free DMEM was used as control (white bars). PDGF-BB was diluted in serum-free DMEM at 10 ng/mL. Columns represent mean number of totally migrated cells, bars represent SEM from three independent donors.

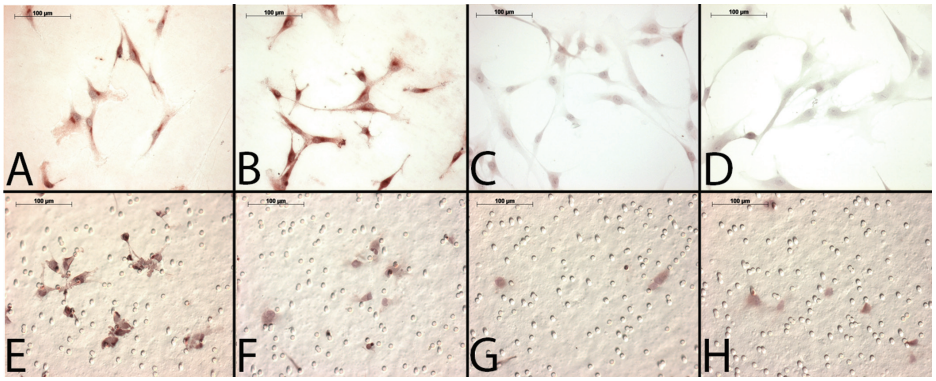

Figure 5. Expression of IL-1R1 in MSC and osteoblasts. Immunocytochemistry with an anti-IL-1R1 antibody was performed on MSC and osteoblasts cultured on glass slides as well as on migrated MSC and osteoblasts. For *in vitro* basal migration and migration toward PDGF-BB, a modified Boyden chamber with 8-μm polycarbonate filters was used. Positive IL-1R1 staining in cultured MSC (A) and osteoblasts (B). Negative control for cultured MSC (C) and osteoblasts (D). Positive IL-1R1 staining in MSC (E) and osteoblasts (F) that migrated toward PDGF-BB. Positive IL-1R1 staining in MSC (G) and osteoblasts (H) that migrated under basal conditions. Scale bars, 100 μm.

Inhibition of Migration Specific Pathways

As JNK and ERK1/2 were activated rapidly during IL-1β signal transduction, we inhibited these kinases to identify signaling pathways responsible for the IL-1β-induced reduction of osteoblast migration. Figure 7A shows that the ERK1/2 inhibitor PD 98059 was able to completely rescue the IL-1β-induced reduction in migration of osteoblasts without chemoattractant (Figure 7A, a) while the JNK inhibitor could only partly restore basal migration (Figure 7A, b). Figure 7B shows that when adding PDGF-BB as a chemotactic factor, the IL-1β-induced reduction of osteoblast migration toward PDGF-BB was restored through ERK1/2 (Figure 7B, c) and also partially through JNK inhibition (Figure 7B, d). These findings suggest that IL-1β reduces osteoblast migration in an ERK1/2 and JNK dependent manner with ERK 1/2 possibly playing a superior role.

We could not further evaluate if one of the p38 MAPK actually plays a role in the IL-1β mediated inhibition of migration, since the p38 inhibitor SB203580 completely abolished any migration activity of osteoblasts (data not shown) which previously has been observed for several other cell types as well (29). Taking together the phosphorylation profile of MAPK upon IL-1β stimulation and our inhibitor experiments, we conclude that IL-1β reduces osteoblast migration in an ERK1/2- and JNK-dependent manner.

DISCUSSION

Controlled release of IL-1β is necessary for successful fracture repair (3,20). If IL-1β levels are increased permanently, however, severe bone loss can occur, since IL-1β has been shown to stimulate osteoclastogenesis and bone resorption by inducing RANKL secretion and suppressing OPG secretion (25–27). While activation of osteoclasts is a well-studied function of IL-1β, few studies are known concerning the inhibition of bone forming cells such as MSC and osteoblasts through IL-1β. We could show, for the first time, that IL-1β completely abolishes osteoblast migration toward several chemotactic factors if presented in a concentration gradient, while MSC migration was not inhibited through

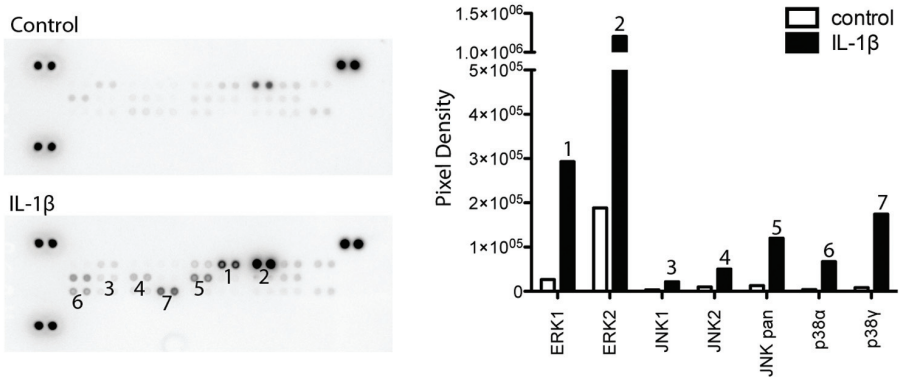

Figure 6. Human Phospho-MAPK Array of control and IL-1β-stimulated osteoblasts. Adherent osteoblasts were left untreated (upper filter, white bars) or stimulated with 100 pg/mL IL-1β (lower filter, black bars) in serum-free DMEM and lysed for analysis with the Human Phospho-MAPK Array.

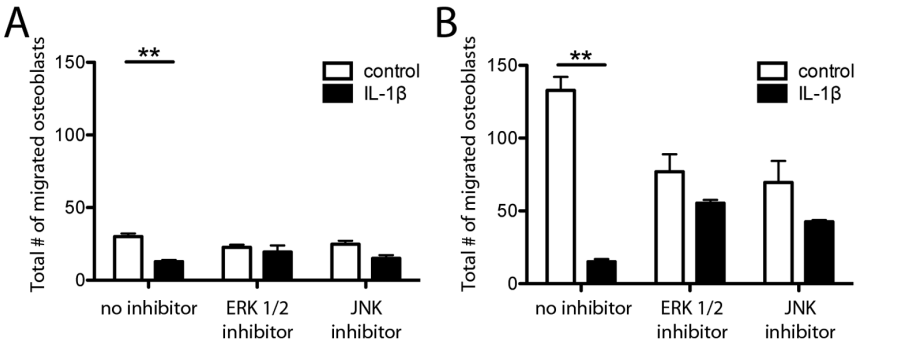

Figure 7. Restoration of IL-1β-induced inhibition of osteoblast basal migration (A) and osteoblast migration toward PDGF-BB (B)—both through inhibition of ERK 1/2 and JNK signaling in osteoblasts. *In vitro* migration was analyzed in a modified Boyden chamber using 8-μm polycarbonate filters. IL-1β was diluted in serum-free DMEM at 100 pg/mL and added to the lower wells of the Boyden chamber as indicated (black bars). Serum-free DMEM was used as control (white bars). PDGF-BB was diluted in serum-free DMEM at 10 ng/mL. For inhibition of chemotaxis with MAPK inhibitors, 10 μmol/L PD 98059 (ERK1/2 inhibition) or JNK inhibitor II was added to upper and lower wells. Columns represent mean number of totally migrated cells, bars represent SEM from three independent donors. Significance was calculated with two-way ANOVA followed by a Bonferroni *post hoc* test; $^{**}P < 0.01$. Inhibition of ERK 1/2 and JNK signaling in osteoblasts in the presence of IL-1β (A) or both IL-1β and PDGFBB (B).

IL-1β. To the best of our knowledge, this is the only situation identified so far in which a concentration gradient of a soluble factor inhibits the directed migration of osteoblasts *in vitro*. We could exclude that the overall process of cell adhesion was compromised under homogenous or gradient exposure to IL-1β. Nevertheless, effects on site-dependent involvement of adhesion receptors, proteases and cytoskeletal dynamics clearly deserve further investigation.

Osteoblasts used in this study are able to migrate nondirectionally and in the direction of a chemotactic gradient as shown previously (6,11,13,19). When exposed to IL-1β, however, osteoblasts' basal migration and migration toward several chemotactic factors was significantly decreased. A study by Gilardetti *et al*. indicated that preincubation of osteoblasts with IL-1β significantly reduces migration toward PDGF-AA by specifically downregulating the binding capacity of osteoblasts for PDGF-AA, but has few effects on the binding of PDGF-BB and migration toward PDGF-BB (30). In another study, IL-1β preincubation was even able to enhance the migratory response of murine osteoblast-like cells toward PDGF-AA (31). The present study shows that, without preincubation, an IL-1β gradient strongly inhibits basal migration of human osteoblasts and completely abolishes the chemotactic function of several growth factors such as PDGF-BB, IGF-1 and VEGF-A, as well as the inflammatory factor C5a *in vitro*. Therefore, we suggest that an IL-1β gradient inhibits more general mechanisms of cell migration in osteoblasts such as expression of migration-relevant adhesion proteins or matrixmetalloproteases. Focal adhesion formation or cytoskeletal rearrangements could also be affected. The signaling mechanism by which IL-1β inhibits migration in osteoblasts, we suggest, depends on ERK1/2 and JNK activation since the IL-1β-induced inhibition of migration could be rescued through ERK1/2 and partially also through JNK inhibitors. Both JNK and ERK1/2 are, among others, known to be central kinases of the IL-1R1 signaling transduction (32) and we found them to be highly phosphorylated upon IL-1β stimulation.

While IL-1β inhibited osteoblast migration, both nondirectional and even more potent in the direction of a chemotactic gradient, we observed that migration of MSC stayed unaffected by IL-1β stimulation *in vitro*. This is supported by a study by Ponte *et al*. in which preincubation of MSC with a ten-fold higher IL-1β concentration did not impact basal MSC migration and migration toward FCS (33). Since MSC used in this study migrated normally in the absence and presence of a PDGF-BB gradient compared with previous studies (6), full migratory function of MSC under exposure to IL-1β can be presumed. Moreover, the positive staining for IL-1R1 indicates that the nonreactive behavior of MSC upon IL-1β exposure in terms of migration does not depend on a lack of IL-1R1 expression.

Inflammatory cytokines including IL-1β, TNF-α and IL-6 are expressed especially during the very early phase after the fracture by immune cells in the fracture hematoma (3,20). A preferential recruitment of MSC instead of osteoblasts in the presence of IL-1β being observed in our study might be important in this early fracture healing stage. In a sheep bone-healing model, for example, more flexible stabilization was associated with a higher inflammatory response, and delayed healing (34).

The importance of IL-1β for bone loss during inflammatory diseases has been shown in various studies as explained before (21–23). The most prominent function of IL-1β in bone loss is the capacity to activate osteoclastogenesis (25–27). We could show in this study that, in addition to promoting bone resorption, IL-1β also seems to influence bone formation processes as it inhibits osteoblast recruitment in the sense of a soluble repellent factor. The IL-1β-concentrations used were within the range of those found in synovial fluid of patients with rheumatoid arthritis and culture supernatant of explanted murine calvaria after implantation of polyethylene or titanium particles indicating physiological relevance (35). Since IL-1β levels in inflammatory bone diseases are elevated over longer periods of time, the balance of bone metabolism might be particularly disturbed by simultaneously elevated bone resorption through osteoclast activation and

impaired osteoblast migration. The combination of both mechanisms may be the central pathogenetic factor for development of osteolytic lesions observed in locally persistent inflammatory situations such as osteomyelitis, subchondral bone lesions in rheumatoid arthritis or periodontal disease.

CONCLUSION

In this study we show, *in vitro*, that chemotactic activity of osteoblasts is reduced in the presence of IL-1β, while MSC stay unaffected. This mechanism may interfere with osteoblast recruitment through osteoclast-mediated release of bone-derived growth factors leading to disturbed coupling of bone formation and bone resorption. The IL-1β-induced inhibition of osteoblast migration was most pronounced in the presence of a concentration gradient indicating that this cytokine acts functionally as a repellent factor. Future studies will have to investigate if more cytokines act as soluble repellent factors on cells of the osteoblast lineage, *in vitro* and *in vivo*, to understand bone remodeling and fracture healing as well as their dysregulation by inflammatory processes in more detail.

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DISCLOSURE

The authors declare they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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