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T-Cell Cytokine Induction of BMP-2 Regulates Human Mesenchymal Stromal Cell Differentiation and Mineralization

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Abstract How T-cells, attracted to local sites of inflammation in arthritides, affect heterotopic ossification is presently unknown. Here, we tested the hypothesis that T-cell cytokines play a role in the differentiation of human mesenchymal stromal cells (HMSC) into the osteoblast phenotype by inducing autologous BMP-2, providing a possible mechanism for heterotopic ossification. HMSC from multiple donor bones were treated with either activated T-cell conditioned medium (ACTTCM) or physiological concentrations of the major inflammatory cytokines, TNF-α, TGF-β, IFN-γ, and IL-17 (TTII), individually or in combinations. ACTTCM induced BMP-2 protein in a time-dependent manner over a 48 h period and alkaline phosphatase (AlkP) within 7 days. In combination, TTII, like ACTTCM, induced AlkP and synergistically induced BMP-2 protein. Either individually, or in combinations of up to three, the T-cell cytokines failed to induce BMP-2 above control levels while a combination of all four cytokines synergistically induced BMP-2 10-fold as assessed by ELISA. TTII induced mineralized matrix as effectively as dexamethasone. Inhibition of p38 MAPK completely inhibited TTII-induced BMP-2 production and matrix mineralization. Real time RT-PCR analysis demonstrated a striking early (within 4 h) increase in *BMP-2* gene expression by TTII, which was suppressed by p38 MAP kinase inhibition. In localized chronic inflammatory diseases, T-cell cytokines released at localized sites of inflammation may be the driving force for differentiation of local mesenchymal stromal cells into the osteoblast phenotype thereby playing a significant role in the heterotopic ossification observed in these diseases. J. Cell. Biochem. 98: 706–714, 2006.

Key words: matrix mineralization; arthritides; heterotopic ossification; MAP kinase

Bone loss associated with postmenopausal osteoporosis [Cenci et al., 2000; Weitzmann et al., 2001a,b; Cenci et al., 2003], spondylarthropathies (SpA) [Huang and Schwarz, 2002], and inflammatory arthritides [Miossec, 2000] is regulated by multiple immune cytokines. Patients with any of these diseases exhibit high bone turnover, defined as accelerated osteoblast activity in association with an even higher osteoclast activity, with the net result of bone loss [Clowes et al., 2005]. As such there is reason to believe that inflammatory cytokines may regulate osteoblast differentiation and bone formation.

Inflammatory cytokines, such as TNF-α have been studied for their role in bone repair. For example, during fracture repair in TNF-α receptor null mice, osteoblasts which are normally recruited into the marrow space are replaced with granulation tissue cells [Gerstenfeld et al., 2001; Kon et al., 2001]. These studies suggest that TNF-α plays a crucial role in promoting postnatal bone repair. Furthermore, recent studies have demonstrated that several cytokines produced by activated T-cells (IL-1, IL-17, TNF-α, and IFN-γ) regulate bone remodeling [van Bezooijen et al., 1999; Chabaud and Miossec, 2001; Miossec, 2004]. In the process of stimulating remodeling multiple T-cell cytokines appear to act in concert to increases bone resorption through osteoclastogenesis and bone formation through activation of osteoblast differentiation [Huang and Schwarz, 2002; Rifas et al., 2003].

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Recent studies have shown that ectopic BMP-2 induces the osteoblast phenotype via a p38 MAP kinase-dependent mechanism in both rodent [Guicheux et al., 2003] and human mesenchymal stromal cells (HMSC) [Noth et al., 2003]. We have previously demonstrated that activated T-cells rapidly induce the osteoblast phenotype in HMSC [Rifas et al., 2003]. Thus, T-cell cytokines released at localized sites of inflammation may be the driving force for differentiation of local mesenchymal stromal cells into the osteoblast phenotype. But how they induce anabolic bone effects and whether they induce bone via induction of BMPs and regulation of p38 MAP kinase is still virtually unknown. We now report that the synergistic actions of activated T-cell cytokines induce HMSC into the osteoblast phenotype via induction of autologous BMP-2 and that this process is regulated by the p38 MAP kinase pathway.

MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich unless otherwise indicated.

Recombinant Cytokines

Recombinant human TNF-α, TGF-β_1, IFN-γ, and IL-17 were obtained from R&D Systems (Minneapolis, MN).

MAP Kinase Inhibitor

The p38 MAP kinase inhibitor, SB203580 was purchased from Calbiochem as the water-soluble form. Stock concentrations of 2 mM of each were prepared and frozen at -20°C until used. Stock concentrations were diluted in medium to 1 μM for the appropriate experiments.

Isolation of T-Cells

Peripheral blood mononuclear cells (PBMC) were obtained in the form of buffy coats from the American Red Cross and further purified by separation on Histopaque (1.077 gms/ml) lymphocyte separation medium as previously described [Rifas and Arackal, 2003]. The Washington University Institutional Review Board approved the use of human buffy coats.

T-Cell Cultures

T-Cells were cultured at 1×10^6 cells/ml in AIM V serum-free medium (GIBCO) and activated as previously described [Rifas and Arackal, 2003; Rifas et al., 2003]. After a 72 h incubation period the activated T-cell conditioned medium (ACTTCM) was harvested and frozen at -80°C until used in the experimental protocols.

Preparation of Human Mesenchymal Stromal Cells (HMSC)

Human rib specimens from six donors were obtained from the Missouri Transplantation Services, St. Louis, MO, as donor tissue. Bone marrow preparations derived from iliac crest of two donors were purchased from Stem Cells Technologies (Vancouver, CA) and one donor from Cambrex BioScience (Walkersville, MD). Bone marrow stromal cells were prepared as previously described [Cheng et al., 1994; Rifas et al., 1995]. HMSC were used at passages 2–3.

Cytokine Stimulation of HMSC

Cells were seeded into wells of 48-well tissue culture plates at 2×10^4 cells/well in Dulbecco's modified Eagle's medium (DMEM, low glucose formulation, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Hyclone) and incubated for 4 days. The confluent cells were washed with phosphate buffered saline (PBS, MediaTech) then incubated for 24 h in DMEM containing 0.2% FBS. Cells were treated with either 25% ACTTCM or T-cell cytokines and the conditioned media (CM) collected after the appropriate time of incubation as noted in the figure legends. To study the regulation of BMP-2 expression by p38 MAP kinase, SB203580, a specific inhibitor of p38 MAP kinase, was added to the cultures at 1 μM for 1 h prior to addition of test agents. Medium was collected after the indicated times and frozen at -80°C until assayed.

BMP ELISA Assays

Production of BMP-2, BMP-4, BMP-6, and BMP-7 in the CM were determined using specific ELISAs from R&D Systems or PeproTech (Rocky Hill, NJ).

Alkaline Phosphatase Assays

HMSC were cultured for 7 days in DMEM, 10% FBS, 10 mM β-glycerophosphate, and 100 μM ascorbic acid 2-phosphate. Cell layers were assayed for alkaline phosphatase (AlkP) as previously described [Rifas et al., 2003].

Alizarin Red Staining

Cells were fixed and stained for alizarin red staining as previously described [Prabhakar et al., 1998]. Alizarin red quantification was performed as previously described [Stanford et al., 1995]. Cells were incubated in DMEM, 10% FBS, 10 mM β-glycerophosphate, and 100 μM ascorbic acid 2-phosphate (Sigma-Aldrich) (differentiation medium) and test agents (TTII or dexamethasone, 100 nM) for 21 days. Medium was changed every 3–4 days and test agents re-added each time. After 21 days, cells were fixed as above then stained in a 1% solution of alizarin red, pH 4.1, for 5 min followed by three rinses with MilliQ water. For some experiments, HMSC were pre-incubated for 1 h with SB203580 then incubated with either medium alone or TTII for 21 days. Medium was changed every 3–4 days with the addition of p38 MAP kinase inhibitor and TTII as indicated.

Quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR)

HMSC were seeded into 100-mm culture dishes (1×10^6 cells/dish) and incubated for 24 h with either medium alone or TTII. Media were aspirated and total RNA extracted using a Qiagen RNeasy mini kit (Qiagen, Inc., Valencia, CA). The isolated RNAs were treated on column with a DNase digestion kit (Qiagen) according to the manufacturer's instructions to eliminate genomic DNA contamination.

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was performed using a QuantiTect SYBR Green RT-PCR Kit (Qiagen). Amplification was performed on 50 ng total RNA per sample using the following primers: BMP-2: forward [5′-TCA AGC CAA ACA CAA ACA GC-3′], reverse [5′-ACG TCT GAA CAA TGG CAT GA-3′] [Bunger et al., 2003]; GAPDH: forward [5′-GCT TGT CAT CAA TGG AAA TCC CAT CAC CAT-3′], reverse 5′-CTT GAG GCT GTT GTC ATA CTT CTC ATG GTT-3′] was used as a control to insure that equal amounts of RNA were amplified. RT was performed at 50°C for 30 min to generate first strand cDNA, followed by 95°C for 15 min to inactivate the reverse transcriptase. PCR was performed as follows: denaturation 94°C, 30 s; annealing 60°C, 30 s; elongation 72°C, 1 min. Each reaction was subjected to melting temperature analysis to confirm single amplified products. Real time PCR was performed in triplicate and BMP-2 amplification was normalized to GAPDH. Negative controls consisted of inactivating the reverse transcriptase by boiling for 5 min prior to RT-PCR to insure that any carryover genomic DNA was not being amplified. BMP-2 and GAPDH were amplified over 40 cycles using a Stratagene MX 4000 light cycler. Data were analyzed using the ΔΔCt method.

Statistics Analysis

Group mean values were compared by Student's two-tailed *t*-test or ANOVA. A *P*-value less than 0.05 was considered significant.

RESULTS

T-Cell Cytokines Induce HMSC Into the Osteoblast Phenotype

We have previously reported that ACTTCM induced HMSC into the osteoblast phenotype [Rifas et al., 2003] and that ACTTCM as well as the synergistic action of four major T-cell cytokines, TNF-α, TGF-β, IFN-γ, and IL-17 (TTII), synergistically induced matrix metalloproteinase-13 in differentiated osteoblasts [Rifas and Arackal, 2003]. In order to determine whether these four cytokines were the active factors in ACTTCM responsible for the induction of HMSC into the osteoblast phenotype, HMSC were treated with either ACTTCM or TTII then analyzed for AlkP activity (Fig. 1). The results show that the TTII cocktail was as effective as ACTTCM in inducing AlkP.

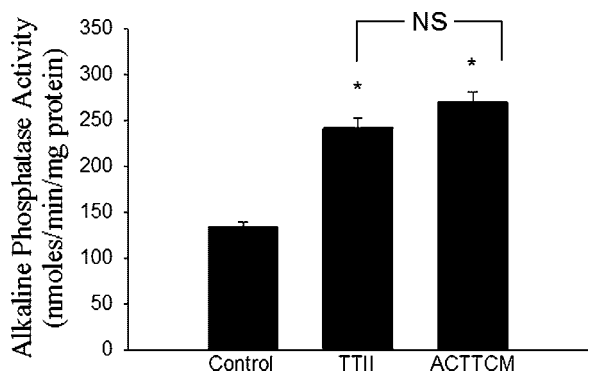

Fig. 1. Induction of alkaline phosphatase (AlkP) by TTII and ACTTCM. Cells were incubated in differentiation medium for 7 days in the absence (control) or presence of the cytokine cocktail (TTII) or 25% activated T-cell conditioned medium (ACTTCM). Cell layers were analyzed for AlkP as described in Materials and Methods. Data represent mean ± SEM of triplicate cultures. * = $P < 0.001$ versus control. Representative of three separate HMSC preparations. NS, not significantly different.

Activated T-Cells Induce BMP-2 in Human Mesenchymal Stromal Cells

To test the hypothesis that T-cell cytokines induce HMSC into the osteoblast phenotype by inducing autologous BMP-2 production, HMSC were stimulated with 25% ACTTCM over a time course of 0–48 h, then assayed for production of BMP-2 using a specific ELISA (Fig. 2). BMP-2 was not detectable until 16 h after treatment, and had a maximum stimulation at 48 h. To determine whether the cytokine cocktail also induced BMP-2 in a similar fashion as ACTTCM, cells were treated for 0–48 h and the CM assayed for BMP-2 using a specific ELISA (Fig. 2). TTII induced BMP-2 after 16 h, and reached a maximum stimulation at 48 h, exactly as found with ATCCM.

Activated T-Cell Cytokines Synergistically Induce BMP-2, but not BMP-4,-6, or -7 in HMSC

To determine whether any of the individual cytokines in the TTII cocktail was responsible for induction of BMP-2 or whether cooperative or synergistic action was involved, HMSC, were treated for 24 h with either medium alone or recombinant human T-cell cytokines, individually or in combinations of two, three, or all four (Fig. 3). Individually, each cytokine alone did not significantly induce BMP-2 levels above that found in untreated cultures. When tested in combinations of two or three cytokines, no significant induction of BMP-2 was found as well. However, when all four cytokines (TTII) were added to cultures of HMSC, a potent synergistic stimulation of BMP-2 production was found. No BMP-4, -6, or-7 production was detected by ELISA (data not shown).

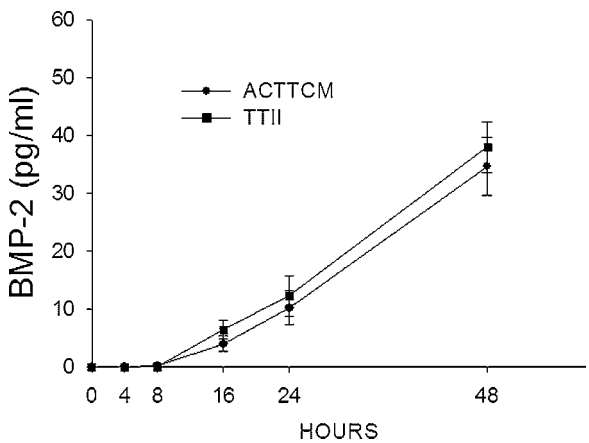

Fig. 2. T-cell cytokines induce BMP-2 in human mesenchymal stromal cells (HMSC). HMSC were treated for 0–48 h as described in Materials and Methods. The conditioned media (CM) were assayed for BMP-2 by a specific ELISA. The T-cell cytokine cocktail TNF-α (120 pg/ml), TGF-β (300 pg/ml), IFN-γ (6 ng/ml), and IL-17 (2 ng/ml) (TTII) induces BMP-2 in an identical fashion as 25% ACTTCM. Both curves are significant, $P < 0.05$ by ANOVA. Data represent the mean ± SEM of duplicate cultures. Representative of three separate experiments.

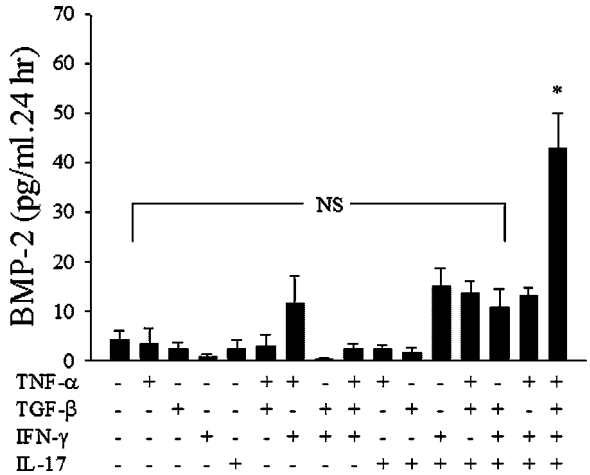

Fig. 3. Effect of T-cell cytokines on BMP-2 induction in HMSC. HMSC were treated with each T-cell cytokine individually, in pairs, triplets, or in quadruplicate for 24 h. The CM were assayed for BMP-2 by a specific ELISA. * = $P < 0.05$ by ANOVA and individual significance determined using Fishers LSD. NS, not significantly different. Data represent the mean ± SEM of two separate HMSC preparations prepared and assayed in triplicate.

TTII Induces Calcification of HMSC

Further analysis of the ability of TTII to induce HMSC into the osteoblast phenotype was performed by incubating the cells in differentiation medium in the absence or presence of TTII or 100 nM dexamethasone (positive control) then staining the cell layers with alizarin red (Fig. 4A). TTII was found to potently induce matrix mineralization to the same extent as dexamethasone. Quantitation of the alizarin red (Fig. 4B) showed that TTII induction of mineralization was identical to that induced by dexamethasone.

Inhibition of p38 MAP Kinase Inhibits T-Cell Cytokine Induction of BMP-2 and HMSC Differentiation

To determine whether the p38 MAP kinase pathway regulated the induction of BMP-2 by the T-cell cytokines, cells were first treated with the specific inhibitor, SB 203580 (1 μM) for 1 h prior to stimulation with TTII (Fig. 5A).

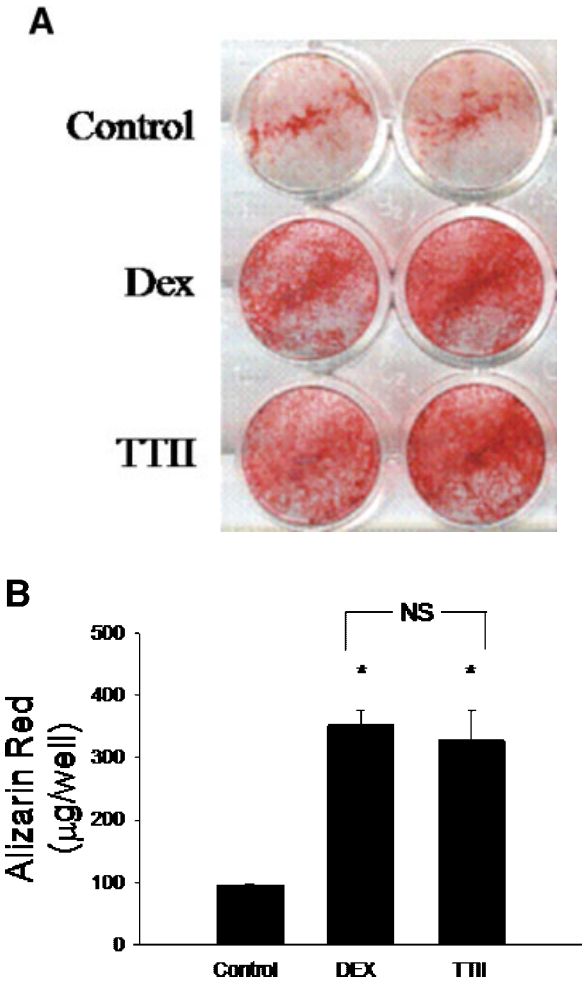

Fig. 4. TTII induces mineralization in HMSC. **A**: HMSC were incubated in differentiation medium for 21 days in the absence (control) or presence of either TTII or 100 nM dexamethasone (DEX) then fixed and stained with alizarin red. **B**: Quantitation of the alizarin red staining in (A). $*P < 0.05$ versus control by ANOVA. Dex versus TTII, not significant. Representative of three separate experiments. NS, not significantly different. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

SB203580 completely inhibited cytokine induction of BMP-2 production. To further examine the effect of p38 MAP kinase on T-cell cytokines induction of differentiation of HMSC into the osteoblast phenotype, cells were treated with either medium alone, TTII, SB203580 (1 μM) or a combination of TTII and SB203580 in the presence of differentiation medium for 21 days. Cultures were stained for calcium deposition with alizarin red (Fig. 5B). In cultures treated with TTII, calcified nodules were formed while no calcification was noted in either control cultures or cultures treated with SB203580 alone or in TTII plus SB203580-treated cultures.

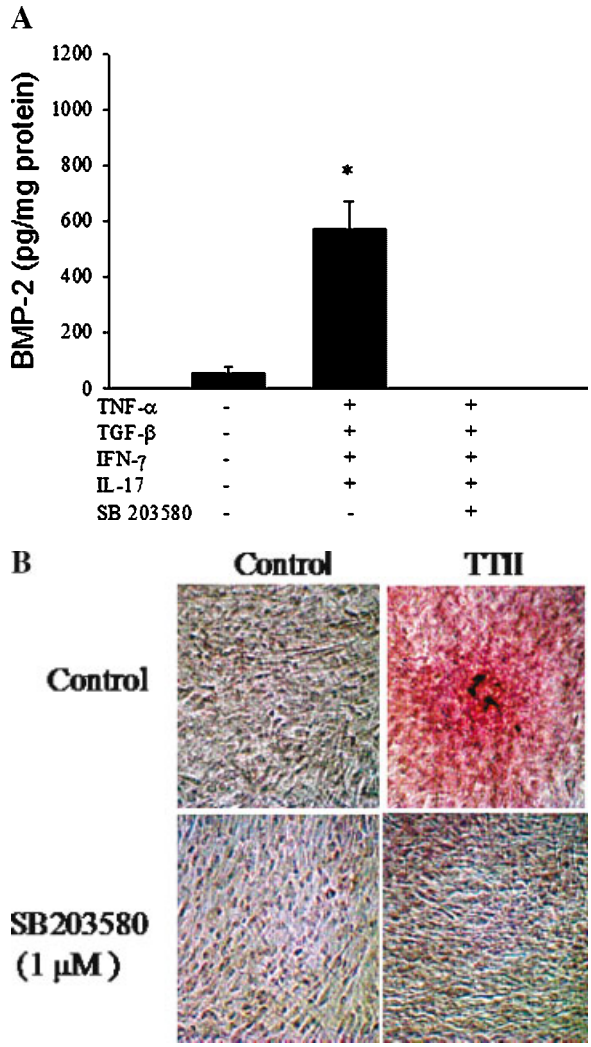

Fig. 5. Inhibition of p38 MAP kinase inhibits TTII induction of BMP-2 and differentiation of HMSC. **A**: HMSC were incubated for 24 h in the absence or presence of TTII or TTII in the presence of the p38 MAP kinase inhibitor SB203580 (1 μM). Media was then assayed for BMP-2 by a specific ELISA. **B**: HMSC were incubated for 21 days in the presence of differentiation medium in the absence or presence of 1 μM SB203580 or TTII or a combination of SB203580 and TTII, then the cultures were fixed and stained with alizarin red to detect calcification. $* = P < 0.001$ versus control or TTII + SB203580 using Student's *t*-test. Data represent the mean ± SEM of two separate HMSC preparations prepared and assayed in triplicate. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

p38 MAP Kinase Regulates *BMP-2* Gene Expression

Having established that TTII induced BMP-2 and that BMP-2 production was responsible for HMSC differentiation into the osteoblast phenotype, we determined whether TTII induced *BMP-2* gene expression and whether that induction was regulated by p38 MAP kinase.

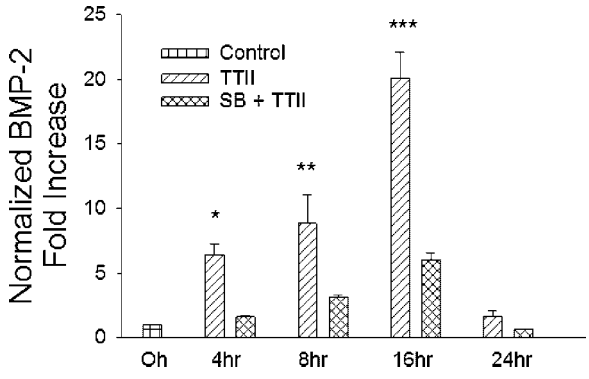

Fig. 6. Inhibition of p38 MAPK inhibits TTII induction of *BMP-2* gene expression. HMSC were incubated in the absence or presence of TTII or TTII in the presence of SB203580 (SB, 1 μM) over a time course of 0–24 h. Data represent the mean ± SEM (n = 3). The induction of BMP-2 mRNA and its inhibition were significant, $P < 0.001$ by ANOVA. Fisher's LSD post-hoc test: * = $P < 0.005$ versus control and <0.007 versus relative SB203580 treatment; ** = $P < 0.005$ versus 0 h relative SB203580 treatment; and *** = $P < 0.001$ versus control or relative SB203580 treatment.

Two separate HMSC preparations were used to analyze both TTII induction of BMP-2 mRNA and the effect of p38 MAP kinase inhibitors. Cells were stimulated with TTII in the absence or presence of either TTII or SB203580 (1 μM), a highly specific inhibitor of p38 MAP kinase, over a time course of 0–24 h and total RNA subjected to qRT-PCR for BMP-2 (Fig. 6). TTII induced significant expression of BMP-2 mRNA within 4 h and attained a maximum level at 16 h before returning to basal levels after 24 h. SB203580 totally inhibited *BMP-2* gene expression over the 4–16 h periods when mRNA was highly expressed. The p38 MAP kinase inhibitor alone did not have an effect on basal *BMP-2* gene expression (data not shown).

DISCUSSION

We have previously reported that the conditioned medium from activated T-cells rapidly induce the differentiation of HMSC into the osteoblast phenotype [Rifas et al., 2003]. We have also reported that the synergistic actions of TNF-α, TGF-β, IFN-γ, and IL-17, four of the many cytokines released by activated T-cells, induce MMP-13 in human osteoblasts via a p38 MAP kinase-dependent mechanism [Rifas and Arackal, 2003]. We now report that these same four T-cell cytokines, acting in a synergistic action, potently regulates the differentiation process of HMSC into the osteoblast phenotype by inducing the autologous differentiation factor, BMP-2. Of particular interest is that individually, the levels of cytokines we found to be secreted by activated T-cells have very little activity in inducing autologous BMP-2 and by extension, do not induce HMSC differentiation alone. This is in sharp contrast to other studies in the rodent system where levels of individual cytokines have been studied at concentrations many fold higher than we find produced by activated human T-cells [Rifas and Arackal, 2003] and have been reported to be negative regulators of differentiation [Spinella-Jaegle et al., 2001; Gilbert et al., 2002]. However, the fact that these four cytokines act synergistically to induce BMP-2 in an identical fashion as that of the crude ACTTCM provides strong evidence that these four cytokines play a major role. The importance of these findings underlies the physiological significance of inflammation in vivo, that is, that activation of T-cells results in the induction and secretion of multiple cytokines and that examination of individual cytokines at high levels, out of context, may lead to erroneous conclusions.

The analysis of the role of IFN-γ in bone remodeling has been mainly restricted to its effect on osteoclast formation and function [Fox and Chambers, 2000; Kamolmatyakul et al., 2001; Yang et al., 2002]. Our studies shed a new light on this cytokine with respect to its role as a critical component of cytokine synergism leading to osteoblast differentiation. Although we have not directly analyzed its function, IFN-γ has been reported to enhance other cytokine actions on transcriptional events through induction of interferon regulatory factor-1 (IRF-1) [Imanishi et al., 2000; Park et al., 2004], again demonstrating the interaction of multiple, low levels of cytokines have a pronounced effect.

We have now shown that BMP-2 can be induced in HMSC by inflammatory T-cell cytokine signaling and that *BMP-2* gene and protein expression are regulated by p38 MAP kinase. Although we have found some variance in the time frame for maximum BMP-2 production in different HMSC preparations (24–48 h) this is not unexpected since we have used primary cultures derived from six different donors. Nonetheless, our data strongly demonstrate the role of inflammatory T-cell cytokines in inducing the osteoblast phenotype.

Our data confirm the work of others [Lee et al., 2002; Noth et al., 2003] who have demonstrated

the role of p38 MAPK in the process of osteoblast differentiation via ectopic administration of BMP-2. However, in those studies, high levels of recombinant BMP-2 were required to induce the osteoblast phenotype. Although we have not examined ectopic administration of recombinant BMP-2, we do find that the production of low levels of BMP-2 by the cocktail of T-cell cytokines is equally effective. We cannot ignore the fact that the amount of BMP-2 measured in the conditioned medium may represent only a portion of the BMP-2 produced by the HMSC and that an unknown amount may have been sequestered either on the cellular membrane or within the extra-cellular matrix. Nonetheless, production of BMP-2 correlates well with the process of HMSC differentiation.

As in the case of ectopic BMP-2 administration, we have found that inhibition of the p38 MAP kinase pathway abrogates the effect of the T-cell cytokine cocktail in inducing the full, mineralizing, osteoblast phenotype. Thus, our findings differ in that we have now demonstrated that T-cell cytokines regulate the induction of autologous BMP-2 and that the levels required are several magnitudes lower than that needed for recombinant BMP-2 to induce osteoblast differentiation and function [Lee et al., 2002; Noth et al., 2003]. Of particular interest is that rodent pre-osteoblasts produce constitutive BMP-2 resulting in extra-cellular mineralization in the presence of ascorbic acid and β-glycerophosphate [Luppen et al., 2003]. In contrast, we have found that HMSC constitutively produce very low levels of BMP-2, but not in sufficient quantities to induce mesenchymal cell differentiation even in the presence of ascorbic acid and β-glycerophosphate. Full differentiation only occurs when HMSC are stimulated by our T-cell cytokine cocktail.

Our data may have significant relevance with regard to diseases in which inflammation plays a role in heterotopic bone formation. One example is the autoimmune disease complex spondylarthropathies, which share clinical features, such as sacroiliitis, axial immobility, and peripheral arthropathies [Huang and Schwarz, 2002]. The pathogenesis of SpA is associated with T-cell recognition of abnormal forms of the human leukocyte antigen HLA-B27 [Boyle et al., 2004], implicating T-cells and antigen-presenting cells in the disease process. Of particular interest is that inflammation precedes the onset of SpA, particularly in the axial skeleton. Furthermore, in SpA, both bone loss and formation occur simultaneously in inflamed regions, suggesting that T-cell cytokines regulate both osteoclast and osteoblast activity. Similar to our studies, proinflammatory T-cell cytokines, such as TNF-α, TGF-β, and IFN-γ appear to play pivotal roles in both formation and resorption in SpA [Huang and Schwarz, 2002]. Presently, the role of IL-17 in SpA is not known but may prove to play a pivotal role. Since both bone formation and bone resorption are high in SpA, it is not unlikely that IL-17 may synergize with other T-cell cytokines to induce osteoblast differentiation while also inducing osteoclastogenesis. In fact, IL-17 acts in a synergistic fashion with TNF-α to promote osteoclast formation and bone resorption [Stamp et al., 2004].

It is becoming increasingly clear that T-cells play a major role in the pathogenesis of Rheumatoid arthritis (RA) [Goronzy and Weyand, 2004]. Interestingly, in RA patients, serum markers of bone formation (osteocalcin (OCN) and bone specific AlkP) and urinary markers of bone resorption (procollagen type I C-terminal telopeptide, pyridinoline and deoxypyridinoline, and hydroxyproline excretion) are significantly higher than those of control patients [Gough et al., 1994; Suzuki et al., 1998]. These biochemical data are consistent with high bone turnover in RA suggesting that the inflammatory process induces enhanced osteoclastic and osteoblastic activity [Gough et al., 1994]. More significantly, reported measurements of cytokines from synovial fluid of RA patients include TNF-α (157 pg/ml), IFN-γ (17–5,677 pg/ml) [Steiner et al., 1999; Cao et al., 2004], TGF-β (110–198.9 pg/ml) [Lettesjo et al., 1998], and IL-17 (12–5,000 pg/ml) [Kotake et al., 1999; Ziolkowska et al., 2000], levels similar to those we have found in 25% ACTTCM [Rifas and Arackal, 2003; Rifas et al., 2003]. These data demonstrate that relatively low levels of cytokines, working in combination can have significant effects in vivo, and support our observations in vitro.

BMPs are the most osteogenic cytokine [Katagiri and Takahashi, 2002] and as such are good candidates for the effect of inflammatory T-cell cytokine induction of mesenchymal stromal cell differentiation into the osteoblast phenotype. In this regard, our data now reveal that inflammatory cytokines, secreted by activated T-cells, work in a synergistic fashion

to induce autologous, localized BMP-2 in mesenchymal stromal cells resulting in their differentiation into the osteoblast phenotype, and opens up a new area of research to examine the effect of localized inflammation on bone formation in chronic inflammatory diseases.

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